DETECTION OF GENOTYPES AND ASSOCIATION WITH INTERLEUKIN 28 B AND TOLL LIKE RECEPTOR 2 POLYMORPHISM IN HEPATITIS C INFECTED PATIENTS

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THESIS
Submitted to
SRI DEVARAJ URS OF HIGHER EDUCATION AND RESEARCH
Kolar, Karnataka for awarding the degree of

DOCTOR OF PHILOSOPHY
IN
MEDICAL MICROBILOGY

Under the faculty of Medicine

UNDER THE GUIDANCE OF

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December 2022

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ACKNOWLEDGEMENTS

Satnam Waheguru

God is one universal creator and we all exist because he has his grace upon us *.So to start with all my heart, I thank god for making me be here and seek his blessings for the time to come as well.*

The potential energy of wood is within the wood; it is released if you know how to rub it. In the same way, the lord's name is within all; the essence is extracted by following the "Guru's" teachings. Every word of this beautiful quote holds true for ***Dr .Prabhakar .K****, whom I got as my mentor and my guide. He deserves to be acknowledged way beyond words. The project is more like a handbook of genres of sir, namely, knowledge, wisdom and excellent ways of execution which I will be taking along and will cherish this lifetime.*

My heartiest gratitude towards my co-supervisor ***Dr. Arvind. N****, who helped and encourages throughout my project.*

I show my thanks to HOD sir ***Dr. Arvind .N*** *for being there to guide me for technical support.*

It's my pleasure to thank honorable chancellor ***Mr.Rajesh N jagadale****. Honorable vice chancellor* ***Dr.Pradeep kumar Goud*** *and respected registar* ***Dr KNV Prasad*** *of* ***SDUAHER f****or the support.*

My sincere gratitude to ***Dr.P.N Sreeamulu****, Professor of general surgery, principal* ***SDUMC*** *and dean –faculty of medicine,* ***SDUAHER*** *for his support and encouragement.*

I am thankful to all the faculties who guided us in the research methodology classes during the first 6 month of PH.D. I am thankful to Ph.D coordinator ***Dr. Kiranmayee.P***

Assoc .Professor, department of cell biology & Molecular genetics for his support in processing our progress report and also supporting for the completion of the project work in genome lab.

I thanks to ***Mr. Ravishanker****, assistant professor, department of community medicine for his help in designing and statistical analysis of the project work.*

I thank to all faculty members, Department of Microbiology, for their support and inputs. A special thanks to my co-Ph.D ***Prathyusha .M, Mr. Vaibhav , Ms Claire Dias*** *and* ***Ms .Mayuri Sukhla*** *for the support throughout the study.*

It said that on earth, people we call god are parents. It very much holds true. Had it not been them, I would never have been able to lead the life which I am blessed with right now, I would owe my life to my parents and feel very lucky to possess a family ***(Mr.Balwant Rai, Mrs.Kailash Rani, Mr. Harbans Lal, Mrs. Sumritra Rani, Mr. Rupinder singh, Mrs .Shivika and Mr. Vishesh Chhabra)*** *which has been unconditionally there throughout thick and thin of my life. For me, there could not be a better heaven up there than what I have already got here in the form of my family.*

I deeply express my thanks to my beloved husband ***Mr .Amrik Kamboj*** *for supporting me financially and mentally in my happy and hard moments. He encourages me to complete my Ph.D project without his support I am unable to reach to this level .Thanks for everthing ....*

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ABBREVIATIONS

HCV	Hepatitis C virus
CHC	Chronic hepatitis C
RT PCR	Real time polymerase chain reaction
RNA	Ribonucleic acid
NK	Natural killer cells
NKT	Natural killer t cells
CTL	Cytotoxic T lymphocytes
TNF	Tissue necrosis factors
IFN	Interferon
PRRs	Pattern recognition receptor
IL	Interleukins
TLRs	Toll like receptors
WHO	World health organization
GWAS	Genome wide association
HAV	Hepatitis A virus
HBV	Hepatitis B virus
NANBH	Non A non B hepatitis
HIV	Human immunodeficiency virus
ORF	Open reading frame

UTR	Untranslated region
NS	Non-structural proteins
ER	Endoplasmic reticulum
EGFR	Epidermal growth factor receptor
LVP	Low viral hepatitis
VLDL	Very low density lipoproteins
HVR	Hyper variable region
FHF	Fulminant hepatitis failure
BMI	Basal metabolic rate
HCC	Hepatocellular carcinoma
IDU	Intravenous drug users
JAK	Janus kinase
STAT	Signal transducer and activator of transcription
ISG	Interferon stimulating genes
NF-κB	Nuclear factor kappa B cells
TIR	Toll/Interleukin-1 receptor
MyD88	Myeloid differentiation pathways
MAPK	Mitogen-activated protein kinase
EIA	Enzyme immunoassay
CIA	Chemiluminescence immunoassay
RIBA	Recombinant immunosorbant assays

NAT	Nucleic acid technique
DNA	Deoxyribonucleic acid
DAA	Direct antiviral agents
DI	Drug interactions
RAS	Resistance-associated substitutes
SNPs	Single nucleotide polymorphism
RFLP	Restriction fragment length

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ABSTRACT

Many forms of liver cancer and cirrhosis can be traced back to the Hepatitis C virus (HCV). The hepatitis C virus infects about 3 percent of the global population. Therefore, HCV infection is considered a major risk to public health. HCV genotypes differ geographically due to structural diversity within the virus's DNA. IL28B polymorphisms influence both the rate of spontaneous clearance and the responsiveness to interferon- (IFN)-based therapy. IL28B is found on Chromosome 19, which encodes interleukin 28B. IL28B is responsible for the production of IFN-3, which has antiviral efficacy against HCV both directly and indirectly through the Janus kinase-signal transducer and activator of transcription (JAK-STAT) complex. Inflammation is triggered when Toll-like receptor 2 binds to the core and NS3 proteins of the Hepatitis C virus. TLR2 maps to human chromosome 4q32. There is evidence from genetic research that TLR2 gene variations influence host defence and disease development. With this research, we hope to evaluate the role of SNPs IL28 B and TLR2 polymorphisms in HCV infection.

The current study's sample size was 248 people, with 124 people serving as HCV-positive patients and 124 serving as healthy controls. Quantitative polymerase chain reaction was utilized to calculate the HCV viral load and genotypes. In order to determine the relationship between the SNPs IL28 B and TLR2 polymorphism, PCR- RFLP -gel electrophoresis was used.

There was a total of 124 HCV-positive patients tested, although only 66 had detectable virus loads. There was no significant difference in the amount of viral RNA between the sexes or age groups. There was a favourable relationship observed between viral RNA levels and liver enzymes. HCV genotype 3 was the most common, followed by genotypes 2, 1, 4, and mixed. Males and younger people tend to have the 3 genotype. There was no statistically significant relationship between viral load and genotyping. The IL28 B rs 126979860 polymorphism and the TLR2 genotype were also studied genetically. The likelihood of being infected with HCV increases when both copies of IL28B contain the minor allele A. Unfortunately, no statistically significant findings regarding TLR 2 polymorphism were found.

In conclusion, among HCV-infected patients, genotype 3 predominated. The polymorphism in SNP rs12979860 was significantly related with HCV infection, while the polymorphism in TLR2 was not. As a result, IL28B can be a therapeutic target, and it may even play a role in vaccine research and development.

CHAPTER I

INTRODUCTION

The presence of HCV infection can be determined using one of several in vitro techniques. The benefits of HCV screening have the potential to be substantial. If HCV infection is caught early, antiviral treatment can be administered earlier in the course of the infection, when it is more likely to be successful (Gupta E et al., 2014). The infection can be identified with preliminary serologic diagnostic tests, but they cannot distinguish between acute and chronic infection. It is typically impossible to discriminate between the acute and chronic phases of hepatitis C infection while afflicted with HCV, despite attempts to do so (Richter SS et al., 2002). Screening for HCV (anti-HCV) antibodies has emerged as the gold standard for diagnosing current and prior infections in clinical labs and blood banks. False positive results, especially in areas with a low frequency of the virus, remain an issue with anti-HCV tests despite progressive increases in sensitivity and specificity (Firdaus R et al., 2015). Virus quantification by RT-PCR and RT-PCR genotyping are the gold standards for diagnosing HCV. When serological tests are positive, a more definitive molecular test for HCV RNA is undertaken to confirm the presence of a current HCV infection (Gupta E et al., 2014).

The innate and adaptive immune responses play crucial roles in preventing and treating viral infections. The human cytomegalovirus (HCV) modifies both the innate and adaptive immune systems. The innate immune response in the liver includes the rapid production of interferon by NK cells, NKT cells, liver macrophages, and infected liver cells. Cellular immune responses, such as CD+4 helper cells (TH) and CD8+cytotoxic T lymphocytes (CTLS), and humoral immune responses, such as antibody-producing B cells (CTLS), make up the

adaptive immune response (Pasha HF, Radwan MI, et al 2013). The complex collection of chemicals known as cytokines play a pivotal role in the launch and maintenance of immune responses. So, they might affect the risk of rapid HCV development. The cytokine family consists of chemokines, interleukins, interferons, and TNFs. Improper cytokine levels appear to affect disease progression, viral persistence, and therapy response in hepatitis C virus infection (Ashfaq UA et al., 2011).

Polymorphism in immune response genes can be used to successfully treat hepatitis C virus (HCV) infection, but it can potentially affect the progression of liver inflammation and cancer. Positively selected receptors (PRRs) generate inflammatory cytokines, interferon (IFN), and other mediators, which engage downstream signaling pathways that activate innate immune responses (Janeway CA et al., 2002). The type III interferon group are a group of anti-viral cytokines, that consists of three IFN-λ (lambda) molecules called IFN-λ1, IFN-λ2, IFN-λ3 (also known as IL29, IL28A, and IL28B respectively) and also referred to as the "type III" IFNs) belong to a new class of IFN related proteins that may have advantages over PEG-IFN for the treatment of HCV (Ge D et al., 2009; Pagliaccetti NE et al., 2016; Robek MD et al., 2010). Numerous cell lines, as well as hepatocytes, basic neuron cells, and alveolar epithelial cells, all express IFN-λ. However, it appears that dendritic cells are the primary cells responsible for producing IFN-λ (DCs) (Ank N et al., 2008). Two recently discovered SNPs, rs12979860C/T and rs8099917T/G, inside the IL28 B gene on chromosome 19 have been found to significantly affect the clinical course of HCV-related-liver

dysfunction and the progression to pegylated-INF-based therapy in HCV infection, particularly chronic HCV infection (Coppola N et al., 2015).

Toll-like receptor (TLR) genetic variation is associated with hepatitis C virus (HCV) infection and severe liver damage. Infection with HCV is linked to many TLRs pathways. There is evidence that the HCV core protein and lipopeptide complexes can activate the innate immune response by binding to Toll-like receptors 2 and 4, respectively. However, this stimulation can lead to an increase in the production of pro-inflammatory cytokines, which can then damage the liver and allow the virus to persist by avoiding the immune system (Neamatallah M et al., 2020). Transcription factor activation and the generation of pro-inflammatory cytokines like interleukin (IL)-6 and IL-8 are two examples of the inflammatory responses triggered by TLR detection of conserved molecular patterns of microbial infections. Genetic studies have uncovered many variants in the TLR2 gene that are linked to host defence and disease development (Akira et al., 2001, Nischalke HD et al., 2012).

CHAPTER II

JUSTIFICATION OF THE STUDY

Hepatitis C can manifest as either an acute or chronic infection of the liver. The prevalence of HCV infection worldwide is estimated to be between 1.8% and 2.6% (Salmani MP et al., 2014) based on research conducted in India. However, there is a lack of data on HCV infection, especially in the Kolar area. We used genotyping and HCV RNA levels to calculate the HCV infection rate in the Kolar area. Because the HCV antibody test suggested by the WHO is known to be unreliable and to routinely generate incorrect findings that inappropriately estimate the prevalence of HCV, the HCV RNA test was used as a confirmatory test.

The distribution of HCV genotypes also varies geographically. In order to design a vaccine that can effectively prevent HCV infection, knowing which HCV genotypes exist is crucial. Additionally, the HCV genotype changes seen in this study may offer researchers with an epidemiological marker that could aid in monitoring the current distribution of HCV genotypes across geographic regions.

Whether or not therapy is used, spontaneous clearance is dependent on host genetic diversity, according to genome-wide association studies (GWAS). Furthermore, polymorphism displays significant inter-population variability. This study was planned to determine the HCV genotypes and also IL28B and TLR2 polymorphism in Kolar region .

CHAPTER III

AIM AND OBJECTIVES

Aim: To determine the HCV genotype and the polymorphism of SNPs *IL28B* and *TLR2* gene in HCV infection, in Kolar population

Objectives:

- To determine the HCV prevalent genotype
- To determine the association of SNPs *IL28B(rs12979860)* with HCV infection
- To determine the association of SNPs *TLR2(-196 to -174 del/ins)* with HCV infection

CHAPTER IV

REVIEW OF LITERATURE

Viral hepatitis was first described by Hippocrates circa 400 BC. However, the causal agents themselves were not identified until the latter half of the twentieth century. Hepatitis is an inflammation of the liver that can be caused by both infectious and non-infectious factors, and it can lead to a wide range of symptoms and complications, some of which can be fatal. Hepatitis virus is classified into five main types: A, B, C, D, and E. Liver disease can be caused by a number of different conditions, each of which is distinct from the others in important ways. Specifically, Types B and C are responsible for the deaths of hundreds of millions of individuals due to chronic liver disease, liver cancer, and viral hepatitis (WHO).

History of HCV

By the 1970s, it was understood that neither hepatitis A nor hepatitis B were responsible for the vast majority of cases of post-transfusion hepatitis. Previously Hepatitis C infection was also known as "non-A, non-B" hepatitis (NANBH). **(**Feinstone SM et al., 1975). In 1988, a group led by Michael Houghton was able to isolate complementary DNA from a patient's blood infected with a "non-A non-B" virus, which paved the way for the isolation of viral RNA and the development of rapid serological diagnostic procedures (Choo QL et al., 1989). Hepatitis C virus (HCV) was not isolated until 1989, although it was quickly recognised as a major causal factor in hepatitis contracted through intimate relationships. Hepatitis C virus, or HCV, is an RNA virus that is part of the family Flaviviridae and the genus Hepacivirus (MS Forman et al., 2011). Viral hepatitis has grown from the tenth to seventh leading cause of death worldwide, surpassing HIV/AIDS, malaria,

and tuberculosis. This is despite the fact that the number of people who are chronic carriers of HCV has fallen from 170 million in 1999 (Cohen J., et al 1999) to 71 million in 2017. (Stanaway J.D., 2013). Some studies have suggested that viral hepatitis is the most common infectious killer worldwide. WHO members states, endorsed with the global hepatitis strategy which aimed to decrease new hepatitis infections by 90% and mortality by 65% (WHO 2017).

Morphology and Structure of HCV

The genome of the HCV virion, which ranges in size from 55 to 65 nm, is 9.6 kb and consists of a lengthy open reading frame (ORF) and an untranslated region on both ends (UTR). Core, E1, E2, and p7 are all structural proteins; NS2, NS3, NS4A, NS4B, NS5A, and NS5B are all non-structural (NS) proteins; and the remaining cleavage products are NS1, NS2, NS3, NS4, and NS5. The high degree of heritability in the HCV genome is an important feature of the virus. The 5' UTR and the 3' UTR's terminal section are both strongly modulated, although the E1 and E2 regions are the most crucial (Lemon SM et al., 2007).

Three structural proteins (Profoundly, E1, and E2) and six non-structural proteins are encoded by the HCV genome, which has an open reading frame (NS2, NS3, NS4a, NS4b, NS5a, and NS5b). In addition, a polypeptide consisting of 30110-11 amino acids is coded (Kato et al., 1995). HCV structural proteins are decoded first because they are encoded by sequences at the 5' end of the genome. Proteolytic cleavage of a polyprotein yields the core E1 and E2 proteins once this region of the genome is articulated in cells (Grakoui et al., 1993), or in reticulocyte lysate

containing microsomal layers (Hijikata et al., 1991; Santolini et al., 1994). In order for the encapsidation of HCV-RNA to take place during infection construction, the capsid protein, which has a size of 21-22 kDa, comprises a few basic (emphatically charged) amino acids at its amino end. HCV's envelope proteins (E1 and E2) are proteins of 31–35 kDa and 68–72 kDa, respectively, that are incorporated into mammalian cells (Santolini et al., 1994). Numerous sites for N-linked glycosylation exist in both E1 and E2.

Around 96% of the HCV genome codes for infection proteins (9033 of the 9397 bases of the HCV genome, with non-coding regions at both the 5' and 3' ends). The 5' UTR is 341-344 bases long, yet it isn't known whether the bases at this end of the RNA atom are changed, for example, by capping with a methyl guanoside build up as in flaviviruses and in eukaryotic mRNAs **(**Choo et al., 1991).

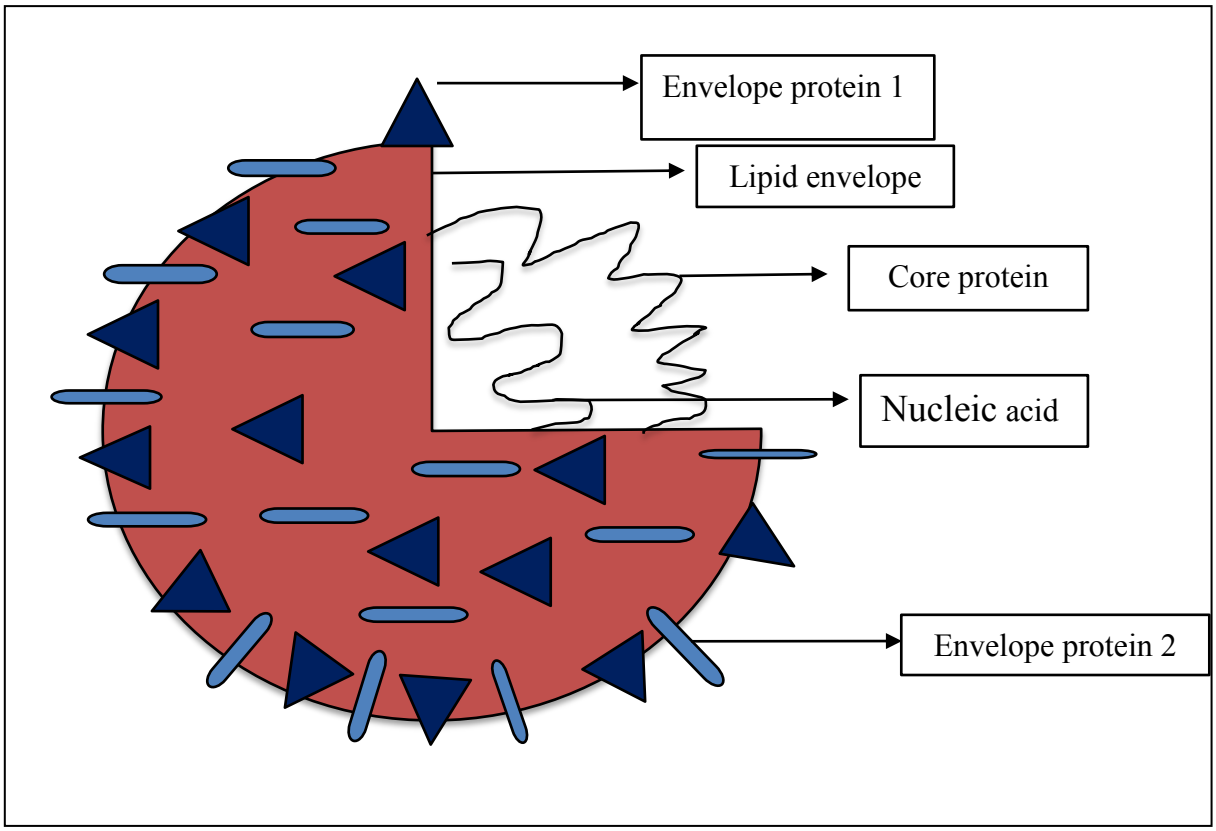


Figure1: HCV virus particle schematic diagram.

HCV Structural proteins

Core protein

The HCV core is a highly conserved basic protein that makes up the viral nucleocapsid. The core of HCV consists of the first 191 amino acids, and it is possible to divide this portion of the virus into three domains based on its hydrophobicity. At the end of p21 is the C-terminus of Domain 2, which is less basic and hydrophobic than Domain 1 (amino acids 1-117). Hydrophobic signal sequence found in domain 3 of the E1 envelope protein (amino acids 171-191) (Bukh J et al., 1994). Gene transcription, lipid metabolism, apoptosis, and other signaling pathways are all influenced by HCV core protein's interactions with a wide range of host cell proteins (Tellinghuisen TL et al., 2002).

Envelope glycoproteins: E1 and E2

HCV comprises two glycoproteins, i.e., envelope 1 (gp35) and envelope 2 (gp70) glycoproteins. These heavily glycosylated proteins serve a crucial function in cell entrance. E1 is the fusogenic subunit of the HCV envelope, while E2 is the receptor-binding subunit. HCV has 4–5 N-linked glycans in its E1 envelope glycoprotein and 11 N-glycosylation sites in its E2 envelope glycoprotein **(**Drummer HE et al., 2003; Goffard A et al., 2005). The number of glycosylation sites, however, varies with genotype. E1 and E2 have highly conserved glycosylation sites with a combination of complicated and high-mannose side chains. HCV glycans are involved in envelope glycoprotein folding, the production of HCV E1E2 complexes, virus-receptor interactions and antigenic diversity (Goffard A et al., 2005**,** Slater-Handshy T et al., 2004).

P7 channel

P7 is a 63-amino-acid polypeptide that is located between the HCV E2 and NS2 genes. P7 is a membrane-spanning protein found in ER (ER). P7 cleavage is mediated by host cell ER signal peptidases. Two transmembrane domains (TMDs) of P7, one of which is oriented toward the ER lumen, are linked by a cytoplasmic loop. P7's carboxyl-terminal TMD has also been shown to function as a signal sequence, facilitating the ER-to-luminal transport of NS2 for proteolytic cleavage by host signal peptidases. In order for a virus to infect a cell, these proteins must first make ion channels. Assembly of viral particles and the release of infectious virions are both genotype-dependent processes that have been shown to need P7 (Griffin SD et al., 2003; Steinmann E. et al, 2007).

Non- structural proteins

The NS2 protein, with a molecular weight of 21-23 kDa, is a transmembrane protein essential for the later stages of the viral replication cycle. To get access to the ER membrane, NS2 forms three or four transmembrane helices at its N-terminus, where it is quite hydrophobic. The C-terminal portion of NS2, which appears to remain in the cytoplasm, plays a crucial role in NS2/NS3 autoprotease activity alongside the N-terminal domain of NS3. The critical area for this cleavage is located between amino acids 827 and 1207 in the C-terminal polyprotein of NS2 **(**Grakoui A et al., 1993; Reed KE et al., 1995, Lorenz IC et al., 2006).

NS3 (P67)

The NS3 protein has multiple roles and weighs 67 kilodaltons. NS3 is a serine protease at its N-terminus and an NTPase/helicase at its C-terminus. It was discovered that the proteins NS3 and NS4A were embedded in the ER membrane. NS3-4A, NS3-4B, NS3-5A, and NS3-5B are all products of cleavage by the N terminus of HCV NS3, which consists of the final 185 amino acids (Wolk B et al., 2000)

NS4A (P8)

The NS4A protein, which has a molecular weight of 8 kilodaltons and a protein sequence consisting of 54 amino acids, is required for the proteolytic activity of the NS3 enzyme. NS4A's N-terminal hydrophobic domain is essential for NS3's ER targeting, as shown by deletion studies. When NS4A and NS5A connect, NS5A gets phosphorylated. Along with its essential role in HCV replication,

NS4A can contribute to viral pathogenesis by influencing a number of cellular activities (Morozov VA et al., 2018).

NS4B (P27)

NS4B is a 27 kDa small hydrophobic protein that is essential for viral protein recruitment. Through its interactions with NS4A, NS4B indirectly influences NS3 and NS5A. The membranous web was formed when NS4B caused structural changes in the ER. All viral proteins were isolated in this region for the formation of replication complexes. (Ashfaq UA et al., 2011).

NS5A (P56\P58)

The molecular weight of the phosphoprotein NS5A is 56 kDa, and its size is 485 amino acids (Figure 2). In HCV-infected cells, both the phosphorylated (56 kDa) and hyperphosphorylated (56 kDa) versions of this protein can be detected (58 kDa). NS5A protein plays multiple roles during viral replication comprising viral genome replication and virus particle assembly (Reyes G.R et al., 2002).

NS5B (P66/68)

NS5B, a protein with 591 amino acids, is found relatively near to the precursor's C terminus. With a GDD motif in its active site, NS5B is an RNA polymerase that requires RNA as a substrate. The antisense strand of HCV RNA is produced by the NS5B protein. According to its crystal structure, NS5B is shaped like a normal 'right hand' polymerase, complete with finger, palm, and thumb domains. A great number of mutations can occur during transcription because NS5B does not "proofread" the RNA. The NS5B protein is being evaluated as a possible target

for antiviral drugs due to its important involvement in virus propagation (De Francesco R et al., 2005; Lesburg CA et al., 1999).

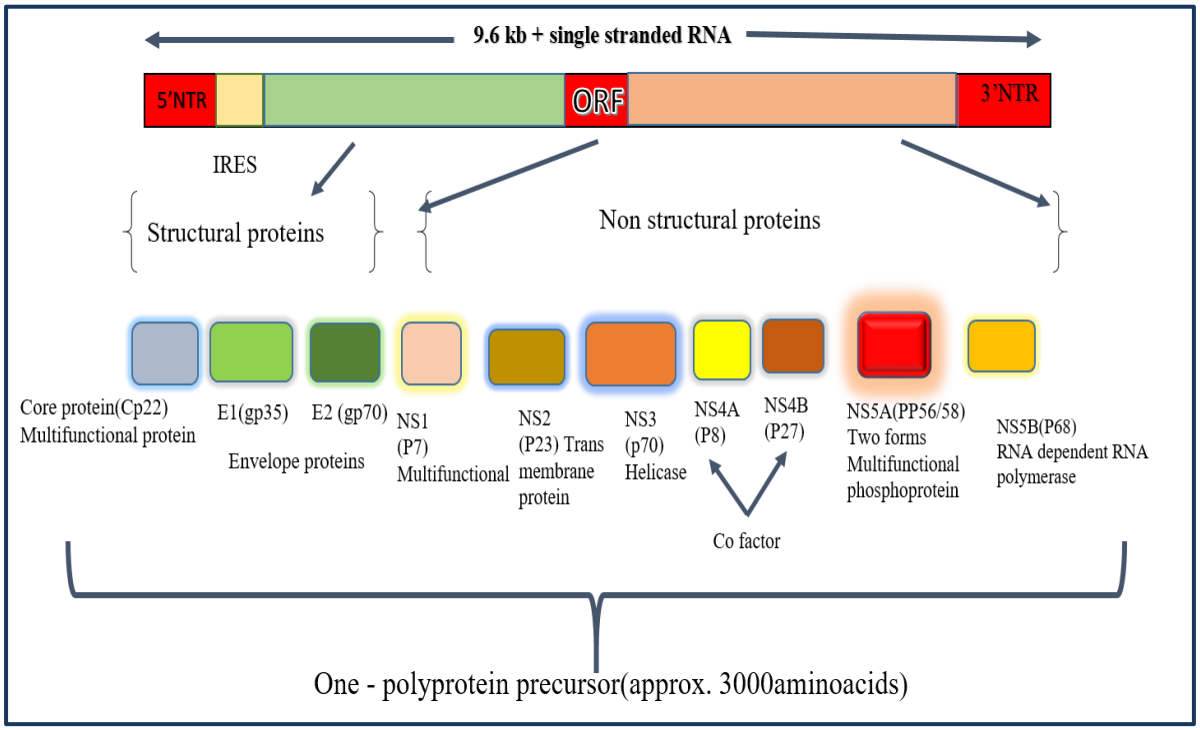


Figure 2: HCV genome encodes proteins**.**

The natural course of HCV

There are two forms of hepatitis infection: acute and chronic. While 50 days is the norm, incubation times can range from 15 to 150 days (Hegedorn et al., 2000). Sixty-five percent to seventy-five percent of people with acute hepatitis have no symptoms at all. Acute hepatitis symptoms should subside, HCV RNA should be undetectable in the blood, and liver enzymes should return to pre-acute levels within 2-12 weeks. The gradual transition from acute sickness to chronic illness after infection is characterized by mild or nonspecific symptoms such as fatigue and malaise (Hoofnagle et al., 1997).

Contrarily, however, 80-85% of those who catch an acute infection will go on to develop a chronic infection. Because of its ability to induce persistent infection through as-yet-unknown mechanisms, Hepatitis C is widely recognised as the primary cause of chronic viral hepatitis in many countries. After an infection has taken hold in a person with a chronic condition, the patient may have mild or nonspecific symptoms including fatigue and malaise. Population-specific factors, including gender, coinfections, and genetics, are known to influence clearance and persistence rates (Thomas DL et al., 2000).

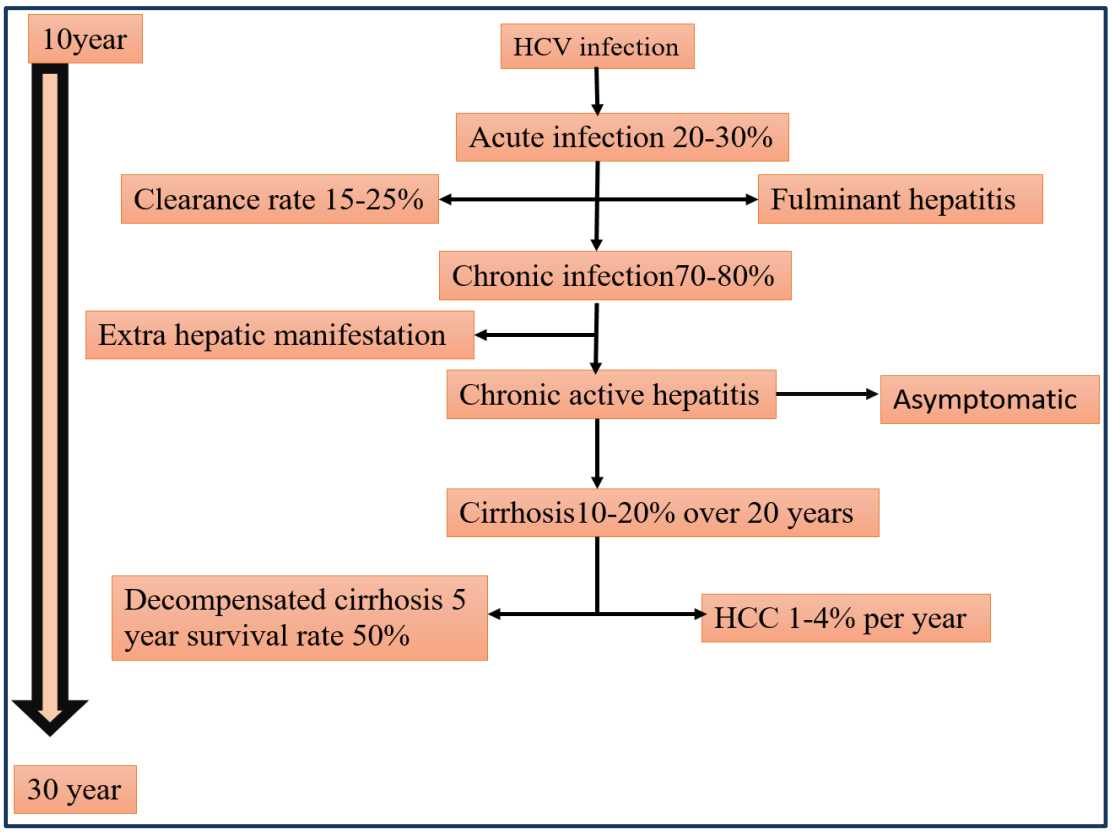


Figure 3: Flow chart representing the natural course of HCV

Replication and life cycle of HCV:

Human hepatitis C virus mostly replicates in cells of the liver called hepatocytes. First, the roles of CD81 and scavenger receptor class B type I (SR-BI) as HCV receptors were determined (Pileri P et al., 1998). The seven processes involved in the reproduction of a virus are shown in Figure 4. The LDL receptor and glycosaminoglycans (GAG) play an initial role in attaching to vulnerable cells before CD81 and SR-BI are involved (Scheele R et al., 2013). At cell junctions, the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN) function as receptors or co-receptors for HCV (Evan MJ et al., 2007; Ploss A., 2009). Cholesterol absorption receptor Niemann-Pick C1-like 1 (NPC1L1) is an essential HCV entry factor (ploss A. 2009). Furthermore, the interaction between CD81 and CLDN1 may be influenced by the epidermal growth factor receptor (EGFR) and ephrin receptor type A2 (Sainz B et al., 2012). For HCV to infect mouse hepatocytes all that is required are the human CD81 and OCLN receptors (Evan MJ et al., 2007). Following receptor engagement and attachment, the HCV genome is released into the cytosol, and the virus is internalised via clathrin-mediated endocytosis and merged with the cellular membrane (Lupberger et al., 2011). Translation, by using IRESs to facilitate translation, the HCV ORF generates a large polyprotein that is then refined into the functional structural and NS proteins. Host signal peptidases in the endoplasmic reticulum process cellular junctions between structural proteins. NS3/4A serine protease and NS2/3 autoprotease (Dubruission J et al., 2008; Choo et al., 1991). The NS proteins of viruses need to be broken down (Moradpour et al., 2007). Replication, NS5B

mediates replication by forming a negative strand intermediate in a replication complex within the ER-derived membranous web. Viruses require a cellular environment with structural similarities to lipid rafts in order to assemble and mature. E1 and E2 envelope glycoprotein heterodimers surround the HCV nucleocapsid in the human cell membrane (Scheel R et al., 2013). The virions form pleomorphic lipoviroparticles (LVPs) with low-density and very-low-density lipoproteins (LDL and VLDL) Viruses are almost certainly releases from cells via excocytosis. (BartenschlagerR,1994).

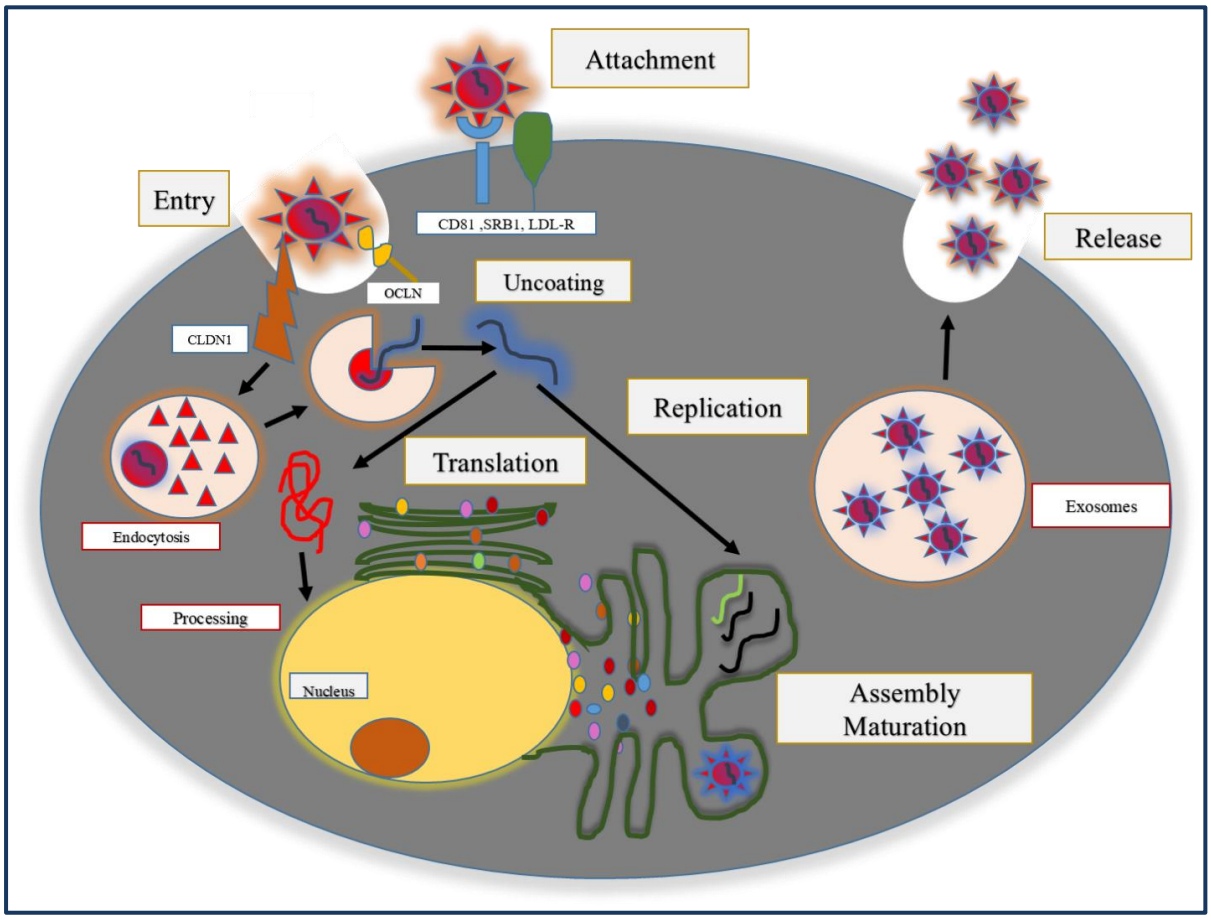


Figure 4: HCV Replication

Variability of HCV genome:

Because its genomic sequence is so similar to that of pestiviruses, HCV has been classified as a member of the Flaviviridae family. The N-terminus location is conserved across structural proteins, while the C-terminus is shared among nonstructural proteins. (Choo et al., 1991). Similar arrangements hold true for the nucleocapsid, envelope glycoprotein, putative protease, helicase, and replicase.

HCV's genetic variation is not uniformly distributed across the virus's genome. Essential viral functions (including translation and replication) and significant structural domains (5'-NTR and 3'-NTR) are correlated with the most conserved areas of the genome. 90% sequence identity across distant strains can be found in the 5'-NTR region of the genome (Menz A et al.2011; Bukh J et al., 1989). In addition, there is high conservation in the region encoding the viral capsid, with 81-88% sequence identity amongst isolates. The most variable part of the genome is found in the coding sequence for the membrane glycoproteins E1 and E2 (Pinerio D et al., 2012). There is the lowest degree of sequence homology (50%) between the various strains in the hypervariable regions 1 and 2 (HVR1 and HVR2) of the E2 gene. There is likely a lot of genetic variation among these viruses because of their large population sizes, short generation times, and fast replication rates (Argentini C et al., 2009).

Viral quasispecies:

Initially identified as a viral quasispecies (Le Guillou et al., 2007), HCV's genomic instability has since been confirmed in multiple ways. In the HCV genome, the NS5B gene, codes for an RNA-dependent RNA polymerase. As the polymerase in HCV lacks a proofreading function, the virus's high defect rate, especially for G:U/U:G mismatches (103 mistakes per site) (Domingo et al., 1997), causes genetic variation and the emergence of quasispecies. The envelope E2 protein's hypervariable region (HVR) was the first genetic marker utilised to distinguish between viral quasispecies. E2's N-terminal region includes HVR-1 and HVR-2, which are located downstream of HVR-1. Mutations to HVR-1, which appears to be the potent epitope, can facilitate viral escape from host immune responses.

Rapid changes in HVR-1 and HVR-2 can provide adaptive benefits on the virus in the form of altered tropism, host range, pathogenicity, or resistance to therapy. An increased rate of amino acid substitutions per site was seen during the acute phase in HCV patients compared to the chronic phase. During chronic infection, one patient (HCV genotype 1b) lost reactivity to the HVR-1(Hyper Variable Region) amino acid sequence, whilst another patient (HCV genotype 2a) retained reactivity to HVR-1. As a result, in HCV infection, HVR-1 may not always express neutralizing epitopes. HVR-1 sequence variants could indicate the presence of several clones in the acute phase, each of which could adapt to generate a persistent, chronic infection (Tsukiyama-Kohara K et al., 2017).

Immune response to HCV:

Hepatitis treatment relies heavily on the inflammatory response as well as the immune system's ability to recognise and destroy virus particles. Proinflammatory cytokines and chemokines are required for the recruitment of innate and adaptive immune cells to the site of infection, where they mature and mount an effective response. Interferons and the innate immune response are the body's initial line of defence against viruses. For effective clearance of an acute viral infection, it is common to need both the innate immune response (interferons, natural killer [NK] cells, and NK T cells) and the adaptive or acquired immune response (CD4+ and CD8+ T cells) that is specific to a given pathogen (Koziel MJ et al., 2005).

Innate immune responses to viral infection are characterized by the fast activation of IFNs and cytokines in humans and other mammals. IFNs inhibit viral multiplication in infected cells and generate an antiviral state in the surrounding uninfected cells by inducing the expression of ISGs with broad antiviral action. Connecting innate and adaptive immune responses, IFNs are also involved in the activation of immunological effector cells (Borden EC et al., 2007). Within 4-8 weeks of infection, the liver becomes a magnet for HCV-specific T cells. Both the cytolytic mechanism and the noncytolytic (IFN-mediated) pathway work to suppress HCV replication. Approximately 30% of people who contract hepatitis C will have their infection cleared up by their immune system during the acute phase (Heim MH et al., 2014). In HCV infection, both innate and acquired immunity plays a vital role. In response to viral infection, natural killer (NK) cells are activated and immature dendritic cells consume viral antigens (iDCs). Once these dendritic cells (DCs) reach maturity, they are able to stimulate CD4+ and NK T

cells. IFN-g is one of many cytokines released by CD4+ cells that stimulates the production of cytotoxic T lymphocytes (CTLs). To control viral replication, CTLs can either lyse infected cells directly or produce cytokines that inhibit viral replication (Koziel MJ et al., 2005)

HCV genotype classification and distributions:

HCV strains are classified into seven recognised genotypes based on phylogenetic and sequencing studies of entire viral genomes (Simmonds P et al., 1994). Within each genotype of HCV, there are 90 verified subtypes and 20 potential subtypes. HCV infections are most common in high-income countries, and researchers have pinpointed subtypes 1a, 1b, 2a, and 3a as the most common worldwide. The uniqueness of the HCV genotype 8 found indicates that the newly revealed lineage is indeed present in the human population (Borgia SM et al., 2018). HCV genotype 1 is the most common (49.1%), followed by genotype 3(17.9%), 4(16.8%) and 2(11%). (Petruzziello A et al., 2016). As a result, genotype 1 accounts for just 31.2% of HCV cases in India, while genotype 3 accounts for 61.8% of HCV cases. Within the Indian population, the occurrences of genotypes 2, 4, 5, and 6 range from 0.05% to 4.5% (Narahari S et al., 2009). According to a survey conducted in the Indian state of Punjab, the third genotype (55.6%) is the most common in the northern region, followed by the first (42.8%), the second (1.6%), and the fourth (1.6%) (Dhiman RK et al., 2016). Genotypes 1 (25.72 percent), 2 (0.002 percent), 4 (7.4 percent), and 6 (2.7 percent) were also found (Christas J et al., 2013).

The prevalence of HCV varies widely not only between regions, but also between individual countries within those regions. Central Asia has the highest prevalence rate, at around 3.6%, followed by Latin America and Western Europe, both at about 0.5%. South Asia and East Asia, both of which have smaller populations than the rest of the world, have the highest infection rates (15.3 and 10.5 million individuals affected, respectively). The current incidence rates are 7.0% in Gabon, 6.4% in Mongolia, and 6.3% in Egypt; the lowest prevalence rate is approximately 0.1% in the Netherlands. Genotype 1 is the most prevalent form of the virus, affecting about half of all infected individuals worldwide (Blach S et al., 2017).

Clinical Manifestation

Most people living with chronic HCV experience no symptoms or rather minor ones, such as fatigue or general malaise. HCV leads to asymptomatic, silent liver disease that progresses slowly until decompensated liver disease and, in rare situations, develops liver cancer. Clinical and pathological features of acute and chronic hepatitis include HCV multiplication in the liver and, presumably, extra hepatic sites, the mechanism of liver destruction, and liver persistence.

Acute hepatitis

Ninety percent of those with acute hepatitis C infection have no symptoms. Some people may have fever, muscle pain, and joint pain. However, jaundice symptoms are not prevalent. It takes about a week or two after exposure for the body to

produce viral RNA, which is the first sign of infection. Seven to eight weeks later, the body's anti-HCV IgG response will become apparent.

Hepatitis C is resolved spontaneously in 20% of cases due to innate and adaptive immunity. The viral RNA is no longer detectable within three to four months after infection. Various factors may facilitate viral clearance. Similarly, severe immune reaction from the host would be indicated by acute hepatitis symptoms. The interleukin (IL) 28B gene polymorphism also affects the host immunological response. It's unusual to see someone with hepatitis C develop fulminant hepatitis (Modi A et al., 2008; Maasoumy B et al., 2012; Thomas DL et al., 2005).

While HCV-related complications are uncommon, fulminant hepatic failure (FHF) can occur. However, with hepatitis C so common, the overall risk of FHF must be fairly low. However, high viral levels may have a role in the development of FHF, especially in those who lack a functional immune system. After 12 weeks of therapy or immunosuppression, patients with chronic hepatitis C often experience fulminant hepatic failure (Funaoka M, et al., 1996; Vento S et al., 2003).

Chronic hepatitis

In around 80% of instances, the immune system is unable to eliminate HCV during the acute phase of infection (Maasoumy B et al., 2012). Viral replication that persists for at least 6 months after an initial infection is considered chronic hepatitis. The vast majority of people who have chronic hepatitis do not have any

symptoms at all. The long-term effects of a chronic infection might vary greatly. Age over 40, male gender, HIV co-infection, higher body mass index, fatty liver, and alcohol usage all hasten disease progression (Thomas DL et al., 2005). After 10-30 years, about 20%-30% of patients develop cirrhosis. Decompensation from portal hypertension in cirrhosis can cause liver failure (ascites, gastrointestinal haemorrhage, etc.). Cirrhotic patients have a 4% annual risk of dying from complications, and a 1-5% annual risk of developing hepatocellular carcinoma (HCC) (Scott JD et al., 2007)

Extra- hepatic manifestation

Although HCV is best known for causing hepatitis, a substantial percentage of chronic HCV patients (40%) also show signs of at least one extrahepatic involvement. The Direct viral cytopathic injury, tissue deposition of host/viral immune complexes, viral-induced immunologic responses like the formation of autoantibodies or activation of specific T lymphocytes, induction of monoclonal or polyclonal lymphocytes, leading to lymphoproliferative disorders, and injury caused by indirect effects of HCV infection are all potential causes of extrahepatic manifestations of HCV infection. Cryoglobulinemia is the condition where this process is most characterised, and it occurs due to the deposit of host or viral immune complexes (Cacoub P et al., 2000).

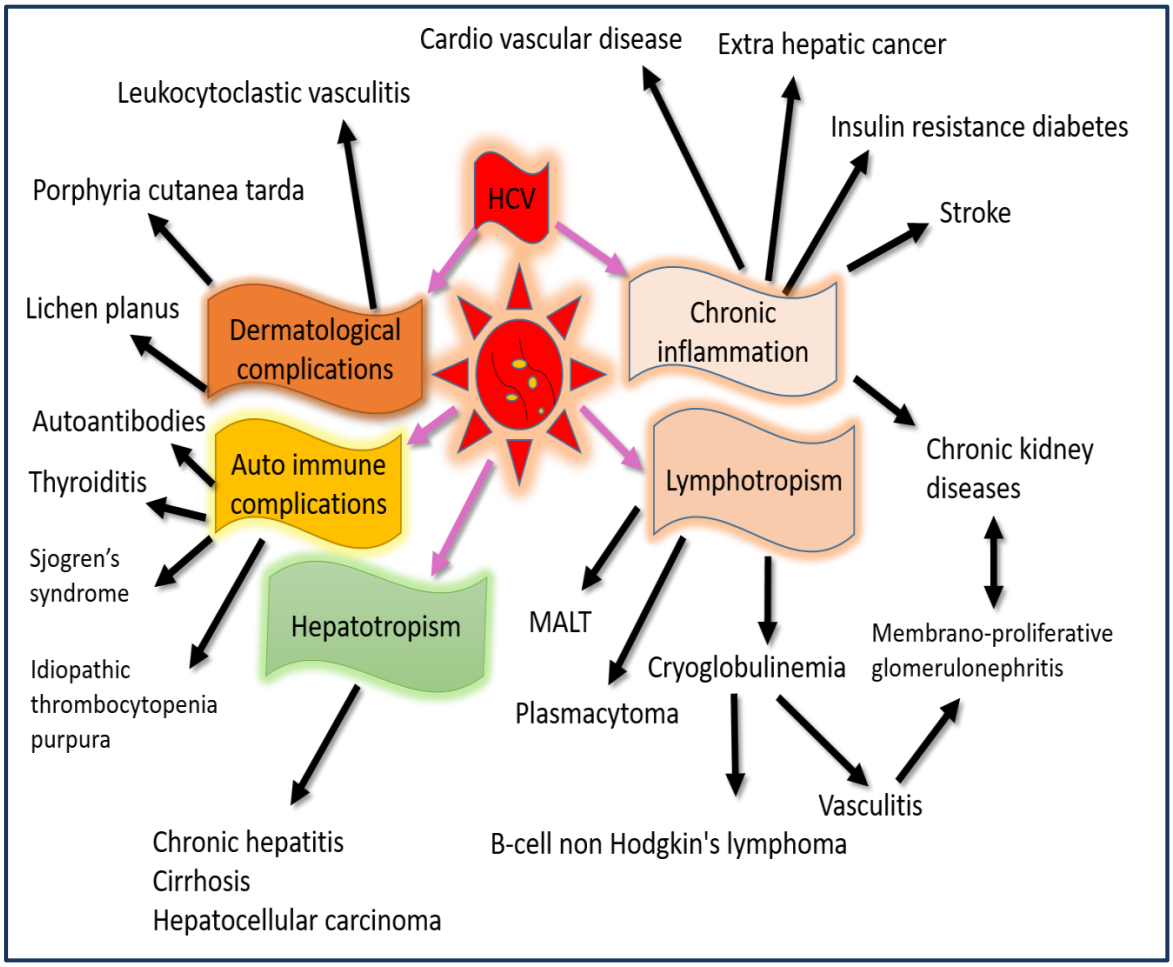


Figure 5: Hepatic and extra-hepatic manifestation

Historical and geographical differences in transmission routes of HCV

People can catch HCV by contact with infected blood. Different modes of exposure to infectious blood have developed over time and across different regions and countries. Before blood screening was widely implemented in the early 1990s, transfusions of tainted blood were the most common route of transmission in high-income countries (Alter MJ et al., 2007; Pepin J et al., 2014; Prati D et al., 2006). Most low- and middle-income countries have instituted blood screening, although transmission remains a problem in many countries' healthcare facilities. Inadequate

techniques of blood screening and the transfer of disease due to the use of unclean medical tools are common in these nations. (Sonderup MW et al., 2017).

Several modes of transmission exist. Unscreened blood products, clotting factors, and other blood transfusions, organ transplants, and the reuse of medical devices in invasive settings (needles, infusion systems, syringes, and catheters) are also potential sources of iatrogenic transmissions (Donahue JG et al.,1992; Pomper GJ et al.,2014 and Dol DE et al., 1986). The transmission of IDU(Intravenous Drug users) HCV can also occur through mother-to-child contact and sexual contact.

Iatrogenic exposures

The transmission of HCV is mostly driven by unsafe therapeutic injections administered by both medical experts and non professionals . Up to 40 percent of all HCV infections globally are thought to be spread through tainted medical injections, which are responsible for approximately 2 million new infections every year (Hauri A.M et al., 2004). In many developing countries, sterile syringe supplies are low or nonexistent, injections are commonly given by people who are not medical experts, and drugs that might be given orally are often given via injection instead. The worst example of iatrogenic transmission of a bloodborne disease was likely caused by the widespread reuse of glass syringes during Egypt's early drive to eradicate schistosomiasis (Frank C et al., 2000). Hepatitis C virus (HCV) may be spread through unsafe injecting practices and improper washing and disinfection of medical and dental equipment.

Prior to the availability of HCV testing, transfusion-related HCV infection posed a serious threat to public health around the world. Despite its near eradication in nations that have instituted routine HCV testing of donors, blood transfusions continue to be a major source of infection in other countries. Inadequate resources and a lack of focus on blood safety mean that certain countries must continue to rely on commercial donors to replenish their blood supply (Busch, M.P et al., 2005; Ladik W et al., 2006).

Injecting drug use has been the main route of transmission in the US for the past 40 years, and it is now responsible for the great majority of newly acquired infections in many other countries, including those in Western, Northern, and Southern Europe. The annual frequency of infection among new injectors remains high, ranging from 15% to > 30%, despite a reduction in the cumulative infection rate from 80% in the late 1980s to 30% in the late 1990s. Transmission of HCV can be sustained with a smaller pool of sharing partners than with other bloodborne viruses, and the virus can be spread through indirect drug sharing and preparation activities such as used cotton swabs (Jarlais DC et al., 2003; Murray JM et al., 2003;Thorpe LE et al., 2002).

Table1: HCV transmission by the grading system

HCV transmission by grading			
Exposure	**Low**	**Moderate**	**High**
Injecting drug use	++++	++	+
Transfusion (unscreened)	+++	+++	+++
Unsafe therapeutic injections	+	++++	++++
Occupational	+	+	+
perinatal	+	+	+
High risk sex	++	+	+/-

Interleukin 28B (IL28B)

The gene structures for interleukin (IL)-28A, -28B, and -29 were found in 2003 (Kotenko SV et al., 2003; Sheppard P et al., 2003). The Nomenclature Committee of the International Society for Interferon and Cytokine Research has classified these newly discovered cytokines as type III IFNs (Ank et al., 2006). The three groups of IFNs are distinguished by their characteristic amino acid sequences and their recognition by distinct receptors. There are three types of interferons: IFN-α, IFN-β, and IFN-λ. Interferon-k3 (IFN-k3) is encoded by the IL28B gene and is a member of the IFN-k family, which also includes IFN-k1 (encoded by IL29) and IFN-k2 (encoded by IL28A). The five-exon human IL28A, IL28B, and IL29 genes are located on the long arm of chromosome 19 at 19q13. By comparing their

amino acid sequences, researchers found that IL-28A and IL-28B are 96% similar and IL-29 is 81% identical (Sheppard P et al., 2003).Their molecular structure, type 3 IFNs belong to the interleukin-10 (IL-10) superfamily, but they're otherwise structurally and functionally quite similar to type 1 IFNs, which are crucial for antiviral action (Witte K et al., 2010).

Directly, type I interferons prevent viral growth in cells; indirectly, they strengthen the body's natural and acquired immune responses, making them better equipped to deal with invading pathogens (Ank et al., 2006).There is a heterodimer between the IL28R and the IL10R that allows IFNs to bind with the two receptor chains. In contrast to the ubiquitous distribution of interferon-receptor (IFNAR) and even interleukin-10 receptor (IL10R), IL28R is exclusive to epithelial cells. Protein tyrosine kinase 2 and the Janus kinase (JAK) are activated when IFN-k binds to this complex, which in turn phosphorylates and activates the signal transducer and activator of transcription (STAT) protein kinases. Through transcriptional activation, phosphorylated STAT proteins (as homodimers or STAT1/STAT2 heterodimers) activate a variety of IFN-stimulated genes involved in immunomodulatory activities (ISGs). Innate defences of cells against viral infection are activated when ISG is increased (Balagopal Aet al., 2010).

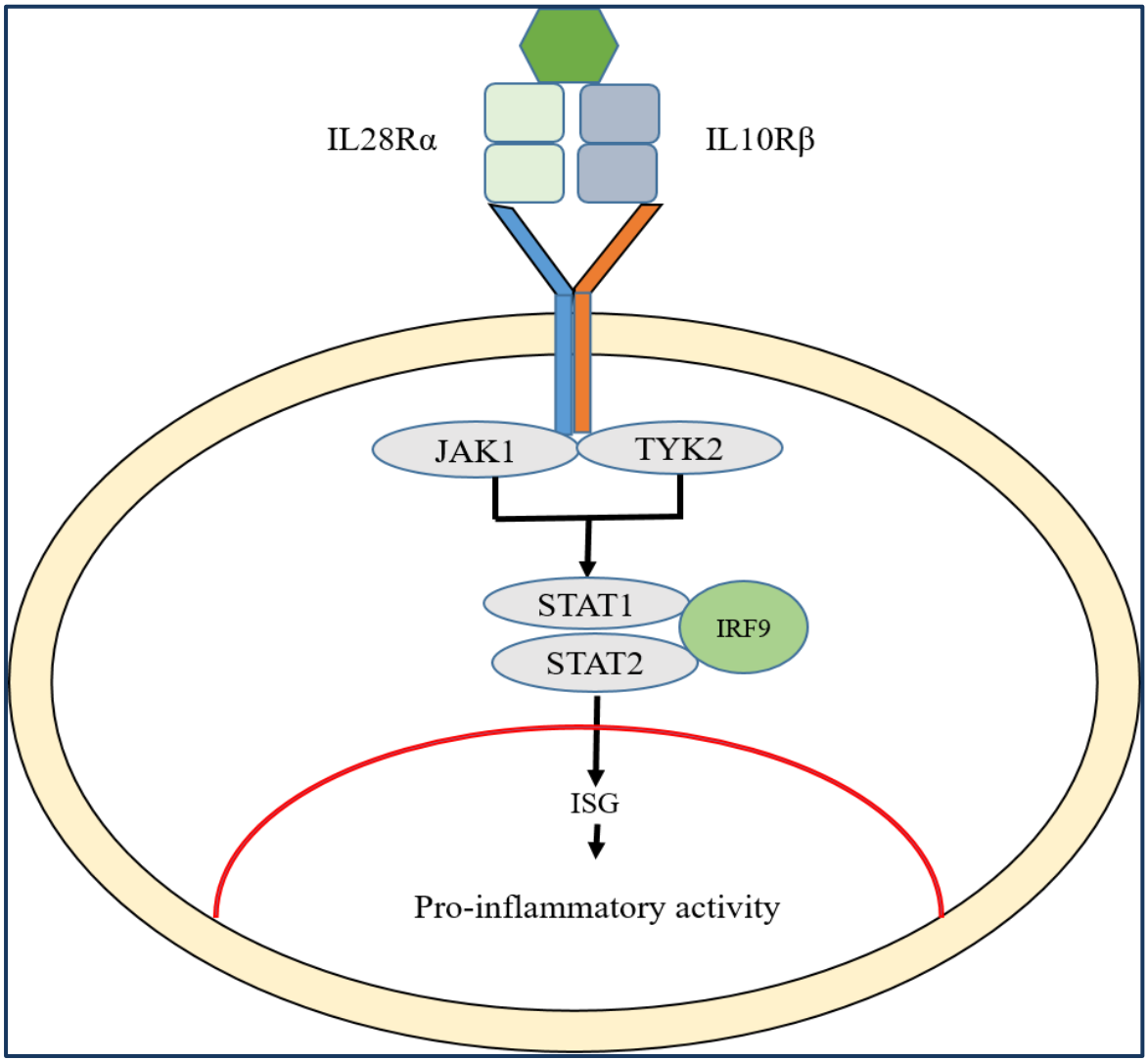


Figure 6: IL28B signalling pathway in HCV

Toll like receptor 2

Toll-like receptors, or TLRs, are a large family of proteins involved in innate immune surveillance (Gitlin L et al., 2006). Toll was discovered to play a role in embryonic dorsoventral patterning in the fruit fly Drosophila melanogaster (Rock FL et al., 1998). Drosophila researchers eventually found that these receptors played a crucial role in the insect's defence system (Anderson KV et al., 1985). Soon after, it was established that animals had similar receptors

to those found in humans (10 of which have been identified so far) (Gitlin, L et al., 2006; Lemaitre, B et al., 1995). Toll-like receptors (TLRs) serve a crucial function in the innate immune system by recognising pathogen-associated molecular patterns from a wide variety of bacteria. By binding to certain adaptor molecules, TLRs activate transcription factors NF-kB and IRFs, which regulate the outcome of innate immune responses. There are ten members of the TLR family in humans (TLR1-TLR10) and twelve in mice (TLR1–TLR9, TLR11–TLR13). In response to pathogen-derived ligands, TLRs (which can be found on the cell surface or in the endosome) start signaling pathways that lead to the production of pro- and anti-inflammatory cytokines (Gitlin L et al., 2006; Barton GM et al., 2002; Ozinsky A et al., 2002; Medzhitov Ret al., 1995). In addition to an intracellular TIR (Toll/Interleukin-1 receptor) domain, these receptors also have an external leucine-rich repeat domain (Ozinsky A et al., 2002). TLRs are expressed by many cell types, both immune and non-immune, including DCs, macrophages, fibroblasts, and epithelial cells. Based on their location within the cell, TLRs are classified as either cell surface TLRs or intracellular TLRs. Cell surface TLRs include TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10; endosomal TLRs include TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13 (Kawasaki T et al.,2014; Celhar T er al., 2012). Different TLRs recruit unique sets of adaptor proteins that share the TIR domain, including MyD88, TRIF, TIRAP/MAL, and TRAM. MyD88 is required for the activation of TLRs and subsequent activation of NF-kB and MAPKs, which in turn activate genes for inflammatory cytokines. Sorting adapter TIRAP binds to TLR2 and TLR4 on the cell surface and thereby recruits MyD88 (Kawasaki T et

al., 2014). MyD88 (Myleid differentiation factor 88)-dependent and TRIF-dependent TLR signaling pathways are differentially expressed. TLR2 heterodimers activate MyD88, which in turn recruits other kinases such mitogen-activated protein kinase (TAK1) and inhibitor of kappa B kinases (IKK). Phosphorylation of the inhibitor of kappa B (IB) by IKKs prevents the NF-B complex from entering the nucleus (Kirschning CJ et al., 1998). Phosphorylated IB is degraded by the proteasome, freeing the NF-B complex to enter the nucleus and activate many genes. According to new studies, the core and NS3 proteins of HCV activate the TLR2-mediated inflammatory cytokine pathway in monocytes and macrophages, hence triggering the innate immune system. The TLR2 pathway was activated by the HCV NS3 and core proteins, but not by the HCV E2 protein, in monocytes and macrophages. Although the core and NS3 proteins of HCV interact with many host proteins (Dolganiuc A et al., 2014; Dahle MK et al., 2015), the methods by which HCV proteins impact the innate immune system to induce illness are not well understood.

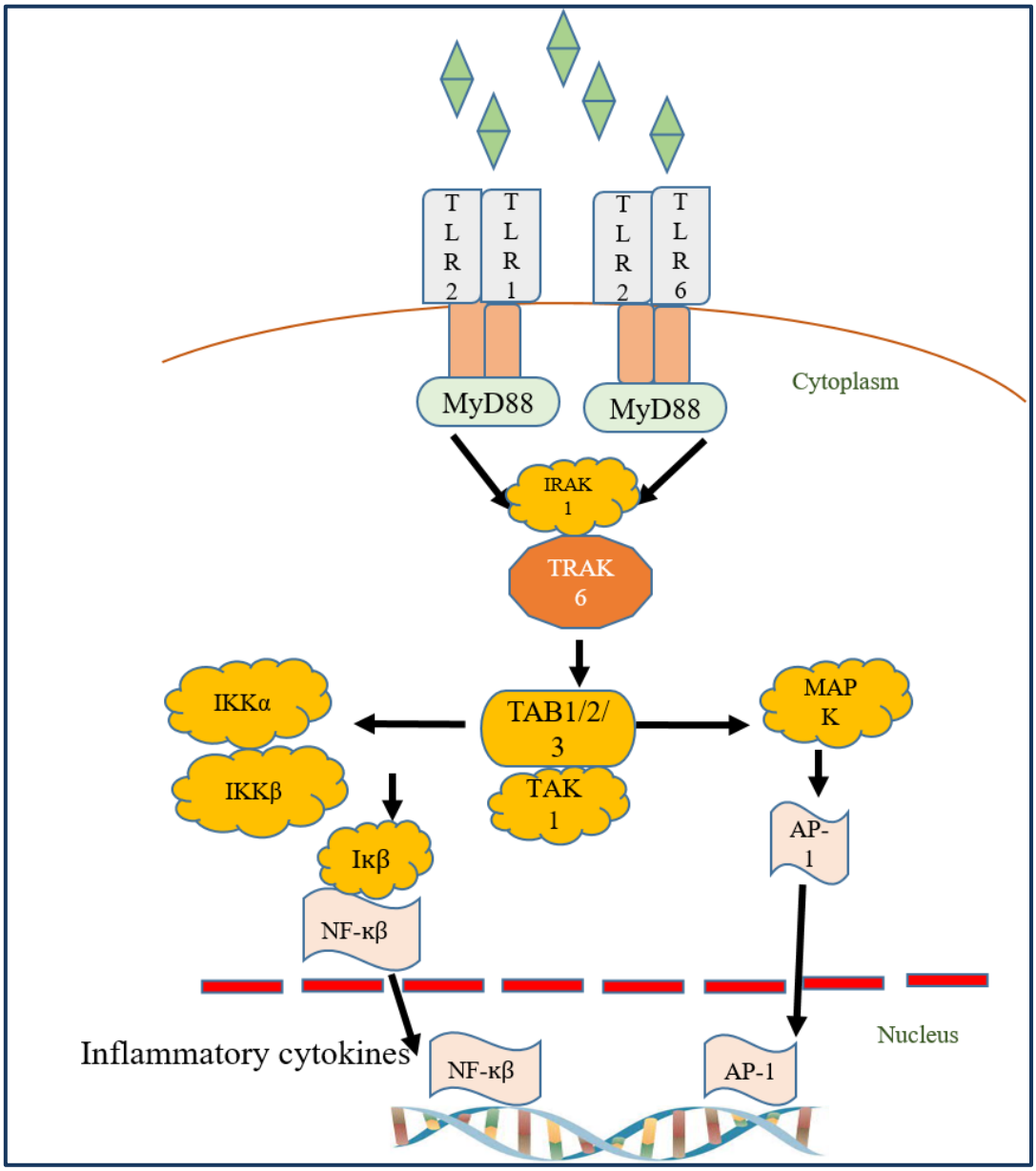


Figure 7: TLR2 signaling pathway in HCV

Laboratory diagnosis

Direct tests that detect, measure, or characterise the components of HCV viral particles, such as HCV RNA and the core antigen, and indirect tests that identify specific antibodies to HCV (anti-HCV), are used to diagnose HCV infection in the lab (direct tests). The time between contracting HCV and having particular

antibodies detected varies from person to person, known as the "serologic window." Seroconversion is detected between 6 and 8 weeks from the beginning of infection using current tests. Direct and indirect virological assays aid in the diagnosis of infections, the selection of appropriate therapies, and the monitoring of viral responses to those therapies**.**

Serological assays:

Serologic methods, such as enzyme-linked immunosorbent assays (ELIAs) for the detection of anti-HCV antibodies and immunoblot tests for the detection of HCV RNA, are generally used to diagnose HCV infection. The development of quick and easy immunoassays has had a significant impact**.**

Rapid Immunoassay and Enzyme Linked Immunosorbent Assays.

Quick immunoassay tests can identify HCV antigens in the core, NS3, NS4, and NS5 regions of the virus. Although these tests are only utilised as point-of-care assays and in conjunction with nucleic acid testing in wealthy countries, they are the primary method of HCV detection in commercial settings in less developed countries. A single drop of whole blood and 20 minutes later, you get your HCV antibody test results with commercially available kits for testing for the virus.

The most common HCV screening tests are those that look for anti-HCV antibodies in the blood (plasma or serum) [enzyme immunoassay (EIA), microparticle EIA, chemiluminescence immunoassay (CIA)]. Since 1989, three generations of enzyme-linked immunosorbent assays (ELISAs) have been

developed to detect anti-HCV antibodies in plasma or serum. These assays use either chemiluminescence or microparticles to increase sensitivity. First-generation tests, which utilised the recombinant c100-3 epitope from the NS4 region, were replaced in 1992 by second-generation assays, which utilised the epitopes c22-3 and c33c from the HCV core and NS3 areas, respectively. Experiments with the third generation included redesigned versions of the core and NS3 antigens, as well as a newly integrated antigen from the NS5 region. When combined with other antigens, overall sensitivity was increased to 97%, surpassing even the performance of the most advanced second-generation diagnostics tools. The mean time to seroconversion for the improved third-generation kits is now only two to four weeks, down from four to six weeks for the older kits (Barrera JM et al., 1995; Morishima C et al., 1999; Colin .C et al., 2001), there is a potential drawback to using EIA approaches: false positive results in healthy individuals and routine blood donors (Chevaliez S et al., 2008).

Recombinant Immunosorbent Assays

In the recombinant immunoblot assay (RIBA), several HCV antibodies are shown as bands on nitrocellulose strips. According to the results of RIBA, those with a positive HCV infection have two reactive bands, whereas those with an intermediate infection have just one. Compared to EIA, RIBA is regarded to be more sensitive because positive cases show two bands. However, as the two tests use similar antigens to detect HCV antibodies, they cannot be called separate gold standards (Damen M et al., 1995; Tobler LH et al., 2001).

Molecular techniques

Nucleic acid technique (NAT) for HCV RNA detection

HCV was one of the first infections to be detected solely through molecular technologies due to the difficulty of growing the virus in cell culture. Molecular virological techniques are important in the diagnosis and monitoring of HCV treatment. NAT is the standard of care for identifying HCV replication. HCV NAT is particularly useful in establishing the diagnosis of acute HCV infection because RNA is detectable as early as 1 week after exposure through needle-stick or blood transfusion and at least 4-6 weeks prior to seroconversion (Maheshwari A ., 2008; Glenn SA et al., 2005; Kamal SM et al., 2008).

Qualitative and quantitative assays are both possible in NAT. Qualitative tests are the gold standard for confirmation. However, the HCV quantitative assay is used to measure the number of international units of HCV RNA per millilitre of serum or plasma (IU/mL) in individuals who are already known to be HCV-positive. The assays have a high dynamic range, enabling for linear quantification up to 107-108 IU/mL and a LOD of 10 IU/mL. The RNA genome of the virus is either destroyed or turned on for use as a standard in quantitative experiments. In order to isolate the target amplicon, the HCV sequences are covered with similar binding sites that serve as HCV RNA targets, and then the specific binding area allows the amplicon to separate the target amplicon. Different genotypes of HCV differ from each other at genomic level ‘The drugs therapy for HCV infection treatment is also dependent on its genotype. Using polymerase chain reaction, the copy number can be

increased, and then amplified (Lee SC et al., 2000). Unlike other methods of amplification, RT-PCR permits continuous monitoring of amplicon kinetics during the exponential phase, well before amplification hits a plateau. In contrast to qualitative PCR assays, when amplicon identification was performed at the very end, this creates a strong relationship between the number of template copies present at the outset (SD Warkad et al.,· 2018).

HCV RNA genotyping assays

Multiple techniques, like as sequencing and hybridization, are used for genotyping (Smith DB et al., 2014). Most genotyping tests first amplify a small region of the viral genome with polymerase chain reaction (PCR), and then sequence the amplified DNA. Nucleotide sequencing is the gold standard for HCV genotyping, while other methods are used. To do this, we can use reverse transcription and polymerase chain reaction to amplify the core (C), envelope (E1), or non-structural (NS5B) regions (Murphy DG et al., 2007; Casanova YS et al 2014). The 5' UTR is the primary focus of most diagnostic tests, while the more genotype-independent core and/or NS5B sections are sequenced in research settings. Genotypes can be very useful in determining the appropriateness of a therapy and the length of a treatment plan (Lole KS et al., 2003; Lole KS et al., 2014; Liu J etv al., 2013).

Treatment

Hepatitis C virus (HCV) infected people receive prompt, top-notch medical attention. In order to stop the course of the disease, screening and treatment are both necessary (Mauss S et al., 2015). Since the development of direct-acting

antivirals, the treatment of HCV has undergone a dramatic shift (DAA). When compared to earlier IFN-based regimens, DAA shows remarkable efficacy in the majority of patients. Patients with decompensated cirrhosis or significant comorbidities, who were previously ineligible for IFN therapy, are now candidates for treatment. Despite advancements in the field as a whole, antiviral treatment for some patient populations is still difficult. Serious side effects from interferon are relatively rare, although they occasionally occur, especially in those with advanced liver disease. Since DAA treatment is generally accepted, individuals with more severe disorders may now be treated, making drug interactions (DI) a particularly pressing matter. Finally, resistance-associated substitutions (RAS) may be utilised as second-line antiviral medication for the small percentage of patients who do not respond to DAA therapy (Van.M et al 2019; Smolders E et al., 2015; Höner Zu Siederdissen et al., 2019). Because of its antiviral and immune-stimulating actions, interferon has been the primary treatment for persistent HCV infection for over 20 years. As a result of pegylation, injections were reduced from every three weeks to once a week beginning in 1997, and the inclusion of the nucleoside ribavirin in the early 1990s greatly enhanced the therapeutic response **(**Pol S., 2019)**.**

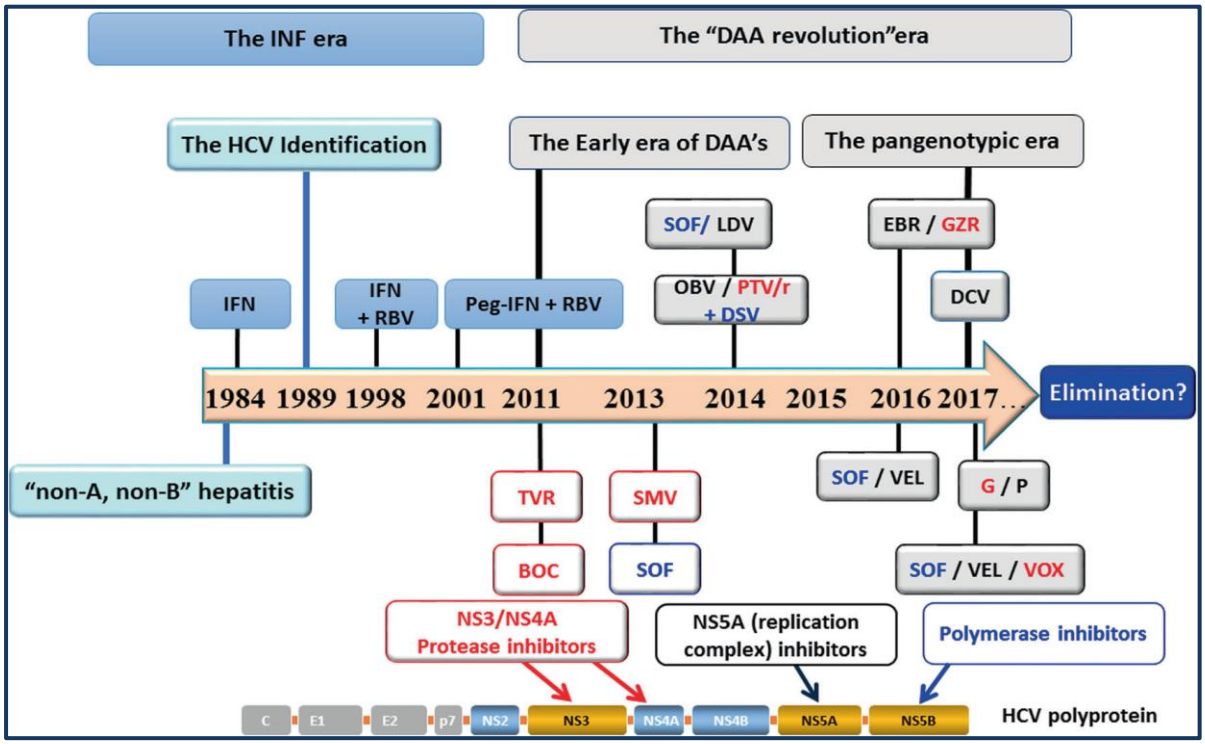


Figure 8: Summary of the hepatitis C virus history and the antiviral treatments against the Hepatitis C virus infection. IFN = Interferon; RBV = Ribavirin; the protease inhibitors are in red (TVR = Telaprevir; BOC = Boceprevir; SMV = Simeprevir; PTV/r = Paritaprevir boosted by ritonavir; GZR = Grazoprevir; G = Glecaprevir; VOX = Voxilaprevir); the polymerase inhibitors NS5B are in yellow (SOF Sofosbuvir; DSV = Dasabuvir) and the replication complex NS5A inhibitors are in white (LDV = Ledipasvir; DCV = Daclatasvir; EBR = Elabsvir; VEL = Velpatasvir; P = Pibentrasvir) .

Source: Pol S, Lagaye S. The remarkable history of the hepatitis C virus. Genes & Immunity. 2019 May;20(5):436-46.

Recent advances in HCV vaccine:

During the past few decades, major advances have been made in the treatment of HCV thanks to the discovery of direct-acting antivirals (DAA) that target HCV nonstructural proteins, which are crucial for viral replication. A new generation of DAA with a cure rate of greater than 95% was created in 2014. However, many obstacles stand in the way of direct antiviral medicines, such as the fact that DAA medications do not prevent re-infections and the prevalence of HCV in underdeveloped countries and among vulnerable persons who have limited access to HCV testing and treatment. These restrictions highlight the critical requirement for a preventative HCV vaccine (Al-Khazraji A., et al 2020; Farci P.,et al 2015 and Cox AL., 2015). A major challenge in creating an effective vaccination against HCV is the virus's considerable genetic diversity.

Vaccines based on cell-mediated and humoral immunity have been developed using a variety of vaccination strategies, such as viral vectors expressing multiple HCV antigens, DNA vaccination (Barnes E.,et al 2012; Folgori A., et al 2006; Li P ., et al 2007), recombinant E2 and E1E2 protein vaccination, and HCV-like virus particles (VLPs) (Ray R ., et al 2010; Logan M., et al., 2017; Wang JA., et al2014; Masavuli MG et al., 2017) and HCV-like virus There are currently three advanced vaccines being tested in human clinical and preclinical trials. Here is a rundown of the several vaccines available:

- A prototype HCV core protein vaccine that has been studied for its ability to trigger T-cell responses in healthy, at-risk individuals is the initial

vaccine candidate being considered. Only 25% of people showed T-cell responses, thus the vaccination was not given to the at-risk population again ISCOMATRIXTM (Drane D et al., 2009).

- The second vaccine recipient will receive an adenovirus-based vector engineered to induce HCV-specific T cell responses. Vaccination experiments in primates led to cross-reactive HCV-specific T cell priming and acute viremia clearance, according to the available data (Meunier JC., et al 2011;Folgori A., et al 2006).
- The third potential vaccine contains recombinant full-length E1E2 envelope (Env) glycoproteins from the HCV-1 strain, which is a genotype 1a strain (Chiron vaccine). Antibody and proliferative T-cell responses against the E1E2 setting were generated in a subsequent phase 1 placebo-controlled clinical trial of the Chiron vaccine (NCT00500747) in healthy human volunteers (Logan M.,et al 2017; Wong JA.,et al 2014; Choo Q. L. 1994; Stamataki Z .,et al 2007; Meunier JC.,et al 2011).

The increasing prevalence of both overt and covert HCV infections highlights the difficulty of combating a human virus in the absence of a vaccination. The development of an effective HCV vaccination would be a major step forward. HCV (Env and nAb) structural, nonstructural, and genomic structures present a potential window of opportunity for developing an HCV vaccine.

CHAPTER V

MATERIALS AND METHODS

Study design:

The present study was a cross-sectional and case-control study carried out between April 2019 to October 2021.The study subjects were enrolled from the Department of General Medicine of R.L jalappa hospital and research Centre, the teaching hospital of Sri Devaraj Urs Medical College, a constituent college of Sri Devaraj university Academy of Higher Education and Research Tamaka, Kolar, Karnataka. The study was carried out following the guidelines of the Declaration of Helsinki and the study was approved by Institutional Ethical Committee (SDUMC/KLR/IEC/467/2019-20). Prior to the recruitment in the study, informed consent was obtained from all the study participants.

The study included 248 subjects and each group included 124 participants. The subjects were divided into HCV infected patients and healthy controls subjects**.**

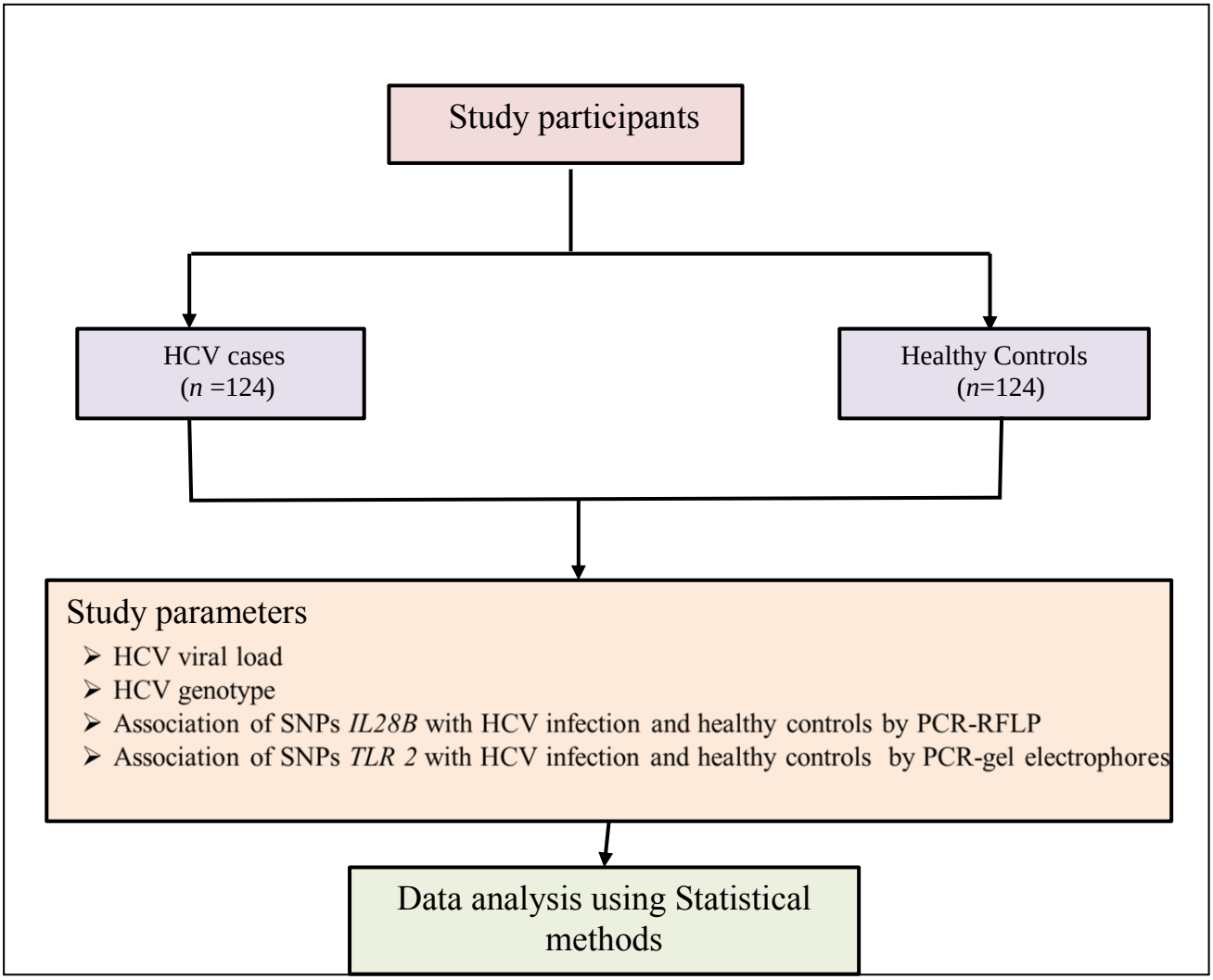


Figure 9: Flow chart of the study design

Patient selection criteria:

Patients were recruited from the Medicine department of the (OP/IP wards) of R. L. Jalappa Hospital and Research Centre, Tamaka, Kolar, attached to Sri Devaraj Urs Medical College.

Table 2: Inclusion and Exclusion criteria

Criteria	Cases	Controls
Inclusion criteria	• Age ≥18 years • Both genders • Symptomatic and asymptomatic HCV infected patients	• Age ≥18 years • Both genders • HCV Negative • Normal AST and ALT levels
Exclusion criteria	• HBsAg positive patients • HIV infected patients • Pregnant women	• History of HCV infection

Sample size calculation:

The sample size was calculated using an open source we- based tool viz., Openepi version 3.0 with 98% confidential interval, and a power of 95%, the number of study participants required for study in each group was found to be 124 (Bhattacharjee D, et al in 2015).

Venous blood (5 ml) was collected from the anticubital vein in plain and EDTA vacutainers. For analysis of biochemical parameters (like liver enzymes AST and ALT) blood sample was collected in tube without anticoagulant.

Estimation of study molecule HCV viral load and genotyping sample was collected in EDTA vacutainers. Serum was used for the estimation of liver enzymes and Plasma was separated for the RNA extraction by using Viral RNA Extraction kit (QIAamp Viral RNA Mini kit, Catalog no. 52904, No. of preparations: 50 reactions) and RNA stored at -20 º for further processing. EDTA Blood with buffy coat was used to extract DNA by using manual method salting out method and then stored at -80º for further processing.

Table 3: Measured biochemical parameters

Sr.no	**Parameters**	**Equipment**	**Reference range**	**Test method**
1.	Alanine transaminase (ALT)(U/L)	Vitro5,1	<50	Multipoint enzymatic by using LDD
2.	Asparate aminoitransferase (AST) (U/L)		14-36	Oxaloacetate decarboxylase pyurayte oxidase/peroxidase

Sample collection:

A total 5ml blood sample was collected of which 2 ml was collected in plain vacutainer and 3ml was in the EDTA vacutainer from all the participants recruited in our study. A consent form was signed by patient. A Proforma was taken from the all the study participants. Sample collection started after getting ethical clearance.

Sample collection & processing:

- 5ml Blood samples was collected by using vein puncture technique.
- Plain and EDTA blood sample was centrifuged at 3000 rpm for 15 minutes.
- Serum and Plasma was separated for biochemical parameters and used for RNA extraction by using Viral RNA Extraction kit (QIAamp Viral RNA Mini kit, Catalog no. 52904, No. of preparations: 50 reactions) and RNA stored at -20 º for further processing.
- EDTA Blood with buffy coat was used to extract DNA by using manual method (Salting out method) and then stored at -80º for further processing.

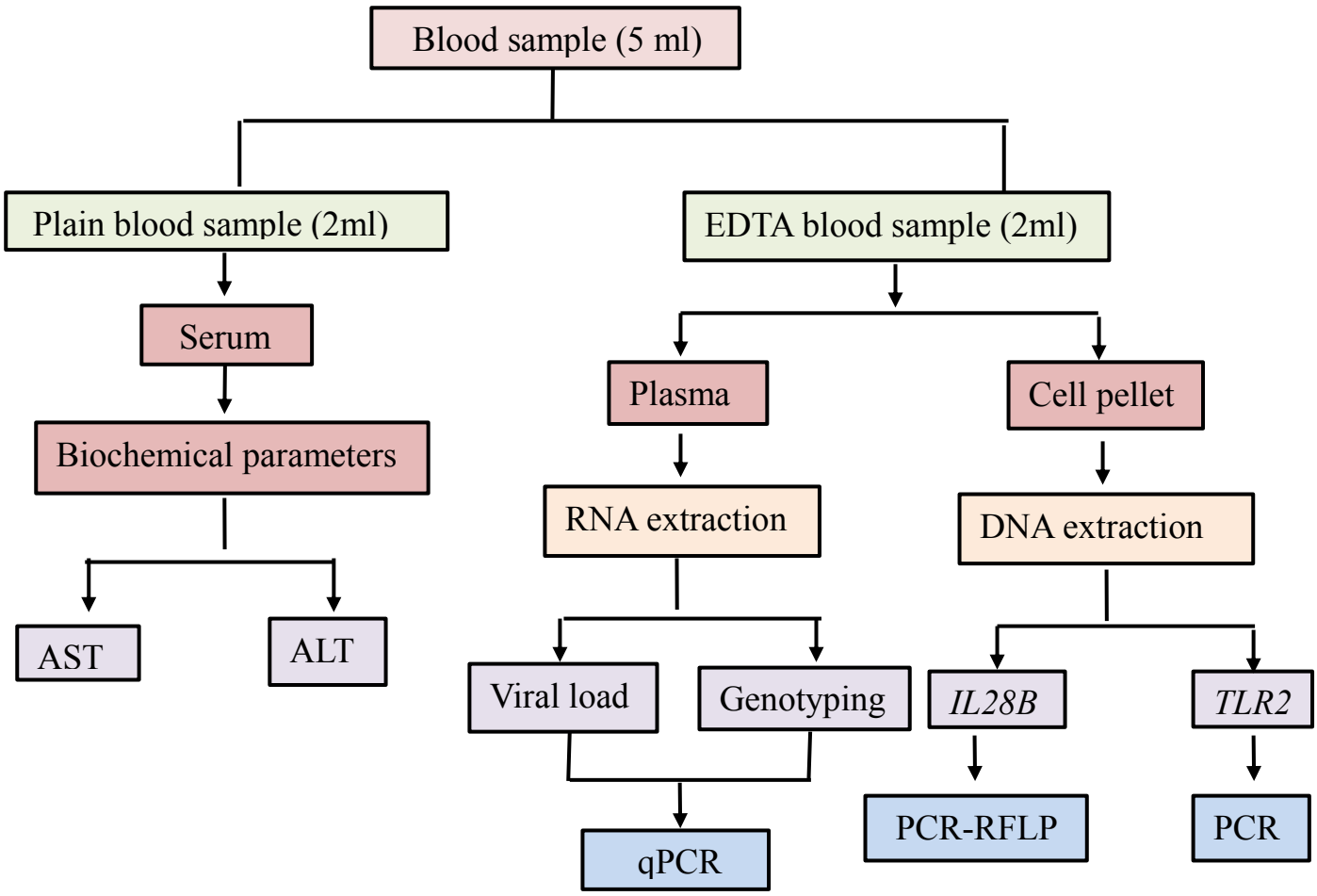


Figure 10: Flow chart of the protocol for HCV positive cases

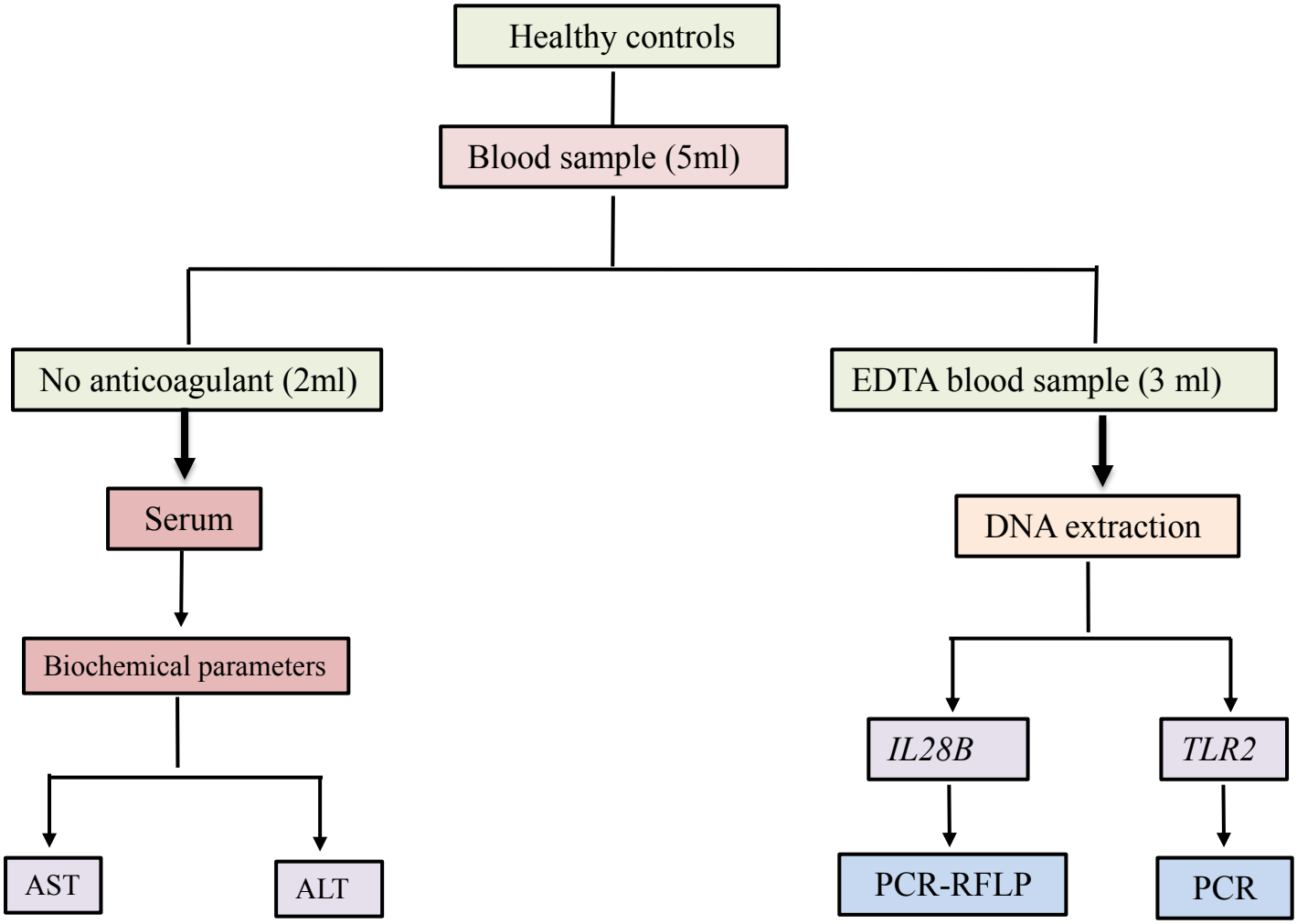


Figure 11: Flow chart protocol for healthy controls

RNA Extraction Protocol:

1. In the 1.5 ml microcentrifuge tube the 560 µl of AVL buffer was prepared containing carrier RNA
2. In the microcentrifuge tube containing buffer AVL-carries RNA 140 µl of plasma was added and then vortex for 15 seconds.
3. The microcentrifuged tube was incubated at room temperature (15–25°C) for 10 min
4. The tube was centrifuged to remove drops from the inside of the lid.
5. In the same tube 560 µl of ethanol (96–100%) was added and vortexed for 15 seconds. After vortexing the tube was centrifuged to remove drops from inside the lid.
6. In the QIAamp Mini column (in a 2 ml collection tube) 630 µl of the lysate solution was added without wetting the rim.
7. The QIAamp Mini column was centrifuged at 6000 x g (8000 rpm) for 1 min.
8. The QIAamp Mini column was placed in the sterile 2 ml collection tube and discard the tube containing the filtrate.
9. In the QIAamp Mini column (in a 2 ml collection tube) 630 µl of the lysate solution was added to the without wetting the rim and centrifuged at 6000 x g (8000 rpm) for 1 min.
10. In the QIAamp Mini column 500 µl of AW1 Buffer was added and centrifuged at 6000 x g (8000 rpm) for 1 min.

11. The QIAamp Mini column was placed in the clean 2 ml collection tube and discard the tube containing the filtrate.
12. In the QIAamp Mini column 500 µl of AW 2 Buffer was added and centrifuged (20,000 x g; 14,000 rpm) for 3 min and dry spin for 1 minute.
13. The QIAamp Mini column was placed into the sterile 1.5 ml microcentrifuge tube.
14. The filtrate from the old collection tube was discarded.
15. In the QIAamp Mini column 60 µl Buffer AVE buffer was added at room temperature.
16. The QIAamp Mini column was incubated at room temperature for 1 min.
17. The QIAamp Mini column was centrifuged at 6000 x g (8000 rpm) for 1 min.
18. In the QIAamp Mini column 60 µl Buffer AVE was added to elute at least 90% of the viral RNA and incubate at room temperature for 1 minute.
19. Viral RNA was stable and then stored at –20°C.

The following protocol was followed for extraction of DNA from the blood buffy coat sample by using manual method: Salting out method (MWer S.,et al 1988**)**

Table 4: Reagent preparation of DNA extraction

Reagent	Composition
Erythrocyte lysate buffer (ELB)	NH_4CL: 8.26 gm
	$KHC_{o3:}$1 gm
	EDTA: 200µl
(20% SDS) Sodium dodecyl sulfate	SDS : 20GM
	Distilled water :100 ml
5M NACL Sodium chloride	NACL : 29.22gm
TE buffer Tris-base acetic acid and EDTA	Distilled water: 100ml
	TRIS: 12.1gm
	Distilled water 100ml pH:7.5
	EDTA:14.6gm
	Distilled water 100ml pH:8.0

Day 1: DNA extraction protocol

1. In a falcon tube, 3ml of EDTA blood was taken.
2. In the falcon tube 1:4 ELB (12mL) was added then vortexed.
3. The Falcon tube was placed in the refrigerator for 30 minutes for hemolysis.
4. The Falcon tube was centrifuged at 1200-3000 rpm for 10 minutes.
5. Without disturbing the pellet, the supernatant was discarded.
6. The falcon tube was inverted on filter paper for drying.
7. To disturb the pellet 2-3 ml ELB was added and make the volume upto 10 ml.

8. The Falcon tube was centrifuged at 1200-3000 rpm for 10 minutes.
9. The supernatant was discarded, to disturb the pellet, 3 ml of ELB was added then shake vigorously to dissolve the pellet and the make volume up to 5 ml.
10. In the falcon tube 270µl of 20% SDS was added to falcon tube.
11. Proteinase K enzyme 30µl was added to the falcon tube.
12. The falcon tube was kept at 37° C in the water bath for overnight incubation.

DAY 2 DNA extraction protocol

1. After overnight incubation the falcon tube was taken out from the water bath.
2. In the falcon tube 500µl of 5M NACL was added.
3. Isopropyl alcohol was added up to marking of 13 ml.
4. The falcon tube was capped firmly screwed and then rinse it gently with slow swirling motion.
5. After this step, DNA from leucocyte lysate begins to appear as a silky mucoid thread consistency. After 20 min swirling motion, DNA get swirled and precipitated in small piece of thread.
6. Transferred the DNA thread into 1.5 ml in Eppendorf tube containing 500µl of frothy prepared 80 % ethanol.
7. The Eppendorf tube was allow to stand for 15 min.
8. The microcentrifuge tube was spined at 12000 rpm for 5min.
9. The supernatant was discarded.

10. In the Eppendorf tube the 500µl of 80 % ethanol was added.
11. The last 2 steps was repeated.
12. The Eppendorf tube was Allow to dry for 15 min.
13. In the Eppendorf tube the 500µl of TE Buffer was added for dissolving the DNA.
14. The Eppendorf tube was incubated at 65˚C for 15 min.
15. The Eppendorf tube was kept on rotaspin for overnight and stored the DNA- 80℃.

Quantitative estimation of viral load by using qPCR (quantitative polymerase chain reaction):

- For the identification of HCV RNA, a quantitative qPCR was carried out using a Magnetic Induction Cycler and the HCV-K-004 kit (CoSara diagnostics. Pvt. Ltd) (Mic-qPCR, biomolecular systems).
- Reagents, a ready-to-use master mix, an Internal Positive Control, and 5 standards were included in the HCV viral load kit. Each set of results was compared with a negative control group that was processed in parallel.
- Quasar 670 dye was used to detect RNA transcribed from the 5' untranslated region of the HCV genome by employing a one-step reverse transcription real-time PCR (Q670).
- To ensure the accuracy of each reaction, a human RNase P gene marker was utilised as an internal positive reference, and CAL Fluor Red 610 dye was used to identify subpar samples (CF610).

- HCV viral load thermal cycling parameters included an activation at 42°C for 2 minutes, a hold at 70°C for 1 minute, a run at 95°C for 20 seconds, and 45 cycles at 95°C for 15 seconds and 55°C for 60 seconds. The total volume of the qPCR reaction was 10 l. Quasar 670 dye was used for detection of the PCR product (Q670).

Detection of HCV genotype by using qPCR:

- HCV RNA positive samples with viral load > 1000 international units per millilitre (IU/mL) were genotyped using Geno Sens. Kit for Detection of HCV Genotypes 1/2/3/4 by Polymerase Chain Reaction (Corbett Research, Australia).
- Add 15 L of RNA for amplification to a total reaction volume of 10 L (Reagent 1: HCV Genotyping Super mix 7.5 L and Reagent 2:Mg. Sol HCV Genotyping 2.5 L).
- A Qiagen Rotor Gene-Q Cycler 5-plex HRM System running software version 2.3.1 was used to evaluate the PCR results.
- The thermal cycling included a first hold at 50 degrees Celsius for 15 minutes (cDNA), a second hold at 95 degrees Celsius for 15 seconds (denaturation), and an annealing step at 55 degrees Celsius for 20 seconds, Extension step 72 degrees Celsius for 15 seconds, then 45 cycles of 95 degrees Celsius for 20 seconds, then 10 seconds, 62 degrees Celsius for 30 seconds, and 56 degrees Celsius for 40 seconds.

- The analysis was completed within three hours by following the instructions.

Genotyping of *IL28B* (rs12979860) Polymorphism

- PCR was used to amplify the reverse complementary region by using a set of specific Forward Primers :5'-CTCAGGGTCAATCACAGAAG-3' and Reverse primers 5'-GAGGATGCAGAGAAGCTG-3'.
- Using online software, the primers flanking the polymorphic region were designed:
  (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi).
- The PCR product was amplified by using total volume of 25 µl comprising 7 µl of distilled water , 1 µl of each forward and reverse primers, a significant amount of 2.5 mm $Mgcl_2$ 1.7 µl 2.5 % of DMSO, 10 µl of Master mix (Ampliqon Taq DNA polymerase 2x master mix catalog number A120301) and 4µl of template DNA .
- The initial denaturation was allowed at 95° C for 5 minutes; then 35 cycles were performed with denaturation of 95° C for 30seconds, annealing at 64.7° C for 30 seconds and extension at 72° C for 1.15 minutes .
- The final extension was carried out at 72° C for 5 minutes.
- Then further 10 µl amplified product was processed for RFLP at 37 ° C for 2 hrs. By using restriction enzyme HPY1II6. A 10 µl amplified product was subjected to 2% electrophoresis to get the bands homozygous genotype

GG 467 bp, 132 bp, homozygous genotype AA 467bp, 102bp and herterozyous genitypeGA 467bp,132 bp and 102 bp fragments.

Genotyping of Toll like Receptor 2 Polymorphism.

- PCR was used for the amplification by using specific Forward primers: 5'CTCGGAGGCAGCGAGAAA 3' and Reverse primer: 5'CTGGGCCGTGCAAAGAAG 3'.
- Using online software, the primers flanking the polymorphic region were designed.

(https://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi).

- The PCR product was amplified by using total volume of 25 µl comprising 9 µl of distilled water , 1 µl of each forward and reverse primers, , 10 µl of Master mix (Ampliqon Taq DNA polymerase 2x master mix catalog number A120301) and 4µl of template DNA .
- The initial denaturation was allowed at 95° C for 5 minutes; then 30 cycles were performed with denaturation of 95° C for 30 seconds, annealing at 59° C for 30 seconds and extension at 72° C for 30 seconds.
- The final extension was carried out at 72° C for 5 minutes.
- A 10 µl amplified product was subjected to 2% electrophoresis to get the bands at homozygous insertion 286bp and homozygous deletion at 264bp .

Statistical analysis:

SPSS 20.0, Graph Pad Prism, and Open epi were used for the statistical analysis. The tests were evaluated to make sure they followed a normal distribution. Statistics were presented as Mean SD for properly distributed data and as Median IQR for data that did not follow a normal distribution. The student t-test was used to evaluate the difference in means between the two groups. Numbers and percentages were used to represent categorical data. To examine categorical variables, researchers utilised Chi-square tests. Statistical significance was assumed at the 0.05 level.

CHAPTER VI

RESULTS

Demographic and biochemical characteristics of the study subjects:

The demographic information of the study participants is summarized in table 5. A total of 248 participants were split evenly between two groups (HCV-positive cases and healthy controls), with 124 people in each.

Table 5: Demographic presentation of participants in the study

Demographic and Biochemical parameters	**Cases (*n*=124)**	**Control (*n*=124)**	***p* value**
Age(years)	51.33±16.5	50.61±16.21	0.80
[#]Gender (M/F)	90(72.6%)/34(27.4%)	83(66.9%)/41(33%)	0.35
AST (U/L)	77.67± 69.75	28.12±9.58	**<0.0001**[**]
ALT (U/L)	74.87±66.72	24.04±6.98	**<0.0001**[**]

The average age of study participants in cases was 51.33 ± 16.5 years and in control group 50.61± 16.21 years (p = 0.80). The frequency was high in males (72.6%, 66.9%) as compared to females (27.4%, 33%) in both the groups. The liver enzymes like AST ($p < 0.0001$) and ALT ($p < 0.0001$) were significantly high in cases group as compared to control group.

Objective 1: To determine prevalent genotype in HCV infected participants

HCV RNA levels among seropositive participants

Viral RNA was extracted from 124 instances with positive Anti-HCV antibodies, and the HCV viral RNA levels were determined using quantitative polymerase chain reaction (qPCR). Of the 124 subjects who tested positive for antibodies against HCV, 58 had undetectable viral loads, while 66 had detectable viremia. Thus, 53.2 % (66/124) of all infections were considered active (figure 12).

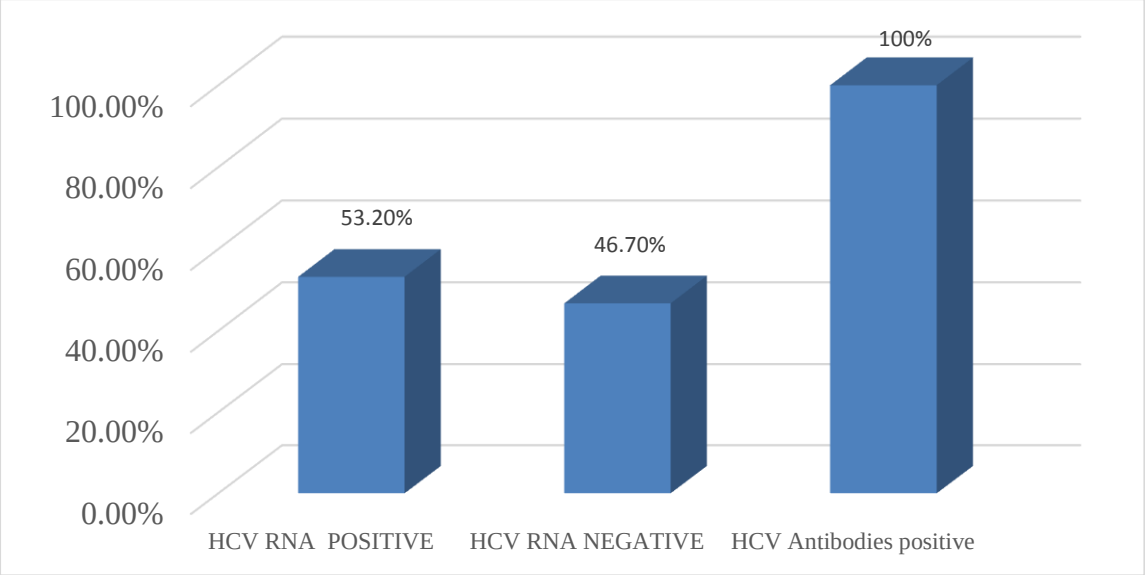


Figure 12: HCV RNA levels among HCV antibody positive

Table 6: Gender and age wise distribution of HCV RNA levels

Variable	Viral load (IU/mL)		*P*- value
	<8,00,000 IU/mL(Low)	**>8,00,000IU/mL(High)**	
Gender			
Male	20(30.3%)	27(40.9%)	0.973
Female	8(12.1%)	11(16.6%)	
Age group			
20-34	3(4.5%)	10(15.1%)	0.070
35-49	6(9%)	13(19.7%)	
50-64	9(13.6%)	10(15.1%)	
65-80	10(15.1%)	4(6%)	
>80	0(0 %)	1(1.5%)	

Anti-HCV antibodies were found in 124 samples, while HCV RNA was found in 66 of those samples (53.22 percent). In 59% (38/66) of the people in the study, a high viral load was found, while in 42.4 % (28/66), a low viral load was found. Based on the research, viral RNA copies were classified as either having a low viral load (800,000 IU/mL) or a high viral load (800,000 IU/mL) (Mishra BK et al., 2020). HCV RNA levels were measured and shown to vary by both sex and age. The ratio of men to females was quite high. High viral loads were seen in

women between the ages of 35 and 49 (16.6%) and 50 and 64 (7.5%), as shown in table 6. Gender and age did not play a significance in the variation in HCV RNA levels.

Table 7: Correlation between viral load and liver enzymes.

Viral load	**AST**		**ALT**	
	R	***P***	***R***	***P***
	0.23	**0.02**	0.35	**0.003**

Correlation of AST and ALT with HCV viral load represent in table 7. The correlation between the viral RNA levels and liver enzyme i.e Aspartate transaminase (r=0.23, p= 0.02) and Alanine transaminase (r=0.35, p=0.003) moderate positive correlation was observed.

Table 8: Clinical details of HCV Infection among 124 Study Participants

Clinical details			**HCV RNA +ve**	**P = value**
Fever	Yes No	0(0%) 124(100%)	0(0%)	<0.00001
Jaundice	Yes no	4(3.2%) 120(96.7%)	0(0%)	<0.00001
Abdominal pain	Yes no	0(0%) 0(0%)	0(0%)	

Acute liver disease	Yes no	15(12.0%) 109(87.9%)	9(7.2%)	<0.00001
Chronic liver disease	Yes no	9(7.2%) 115(92.7%)	9(7.2%)	<0.00001
Cirrhosis	Yes no	11(45.8%) 113(91.1%)	6(4.8%)	<0.00001
Cholelithiasis	Yes no	10 (8.0%) 114(91.9%)	4(3.2%)	<0.00001
Blood transfusion	Yes no	5(4.0%) 119(95.9%)	1(0.8%)	<0.00001
Surgery	Yes no	10(8.0%) 114(91.9%)	5(4.03%)	<0.00001
Metabolic acidosis	Yes no	14(12.0%) 110(88.7%)	1(0.8%)	<0.00001
Fatty liver	Yes no	15(12%) 109(87.9%)	9(7.2)	<0.00001
Dialysis	Yes No	8(6.4%) 116(93.5%)	7(5.6%)	<0.00001
Drug abuse	Yes No	0(0%) 0(0%)	0(0%)	
others	Yes No	16(12.9%) 118(95.1%)	15(12%)	<0.00001

A comparison of clinical details of acquiring HCV infection among participants has also been recorded (Table 8). It was observed that participants with clinical history of fever, jaundice, ALD, CLD, Cirrhosis, cholelithiasis, blood transfusion, surgery, metabolic acidosis, fatty liver, dialysis, drug abuse had significant (p = 0.0001) chance of having HCV infection. Those with no educational background were more likely to have HCV infection.

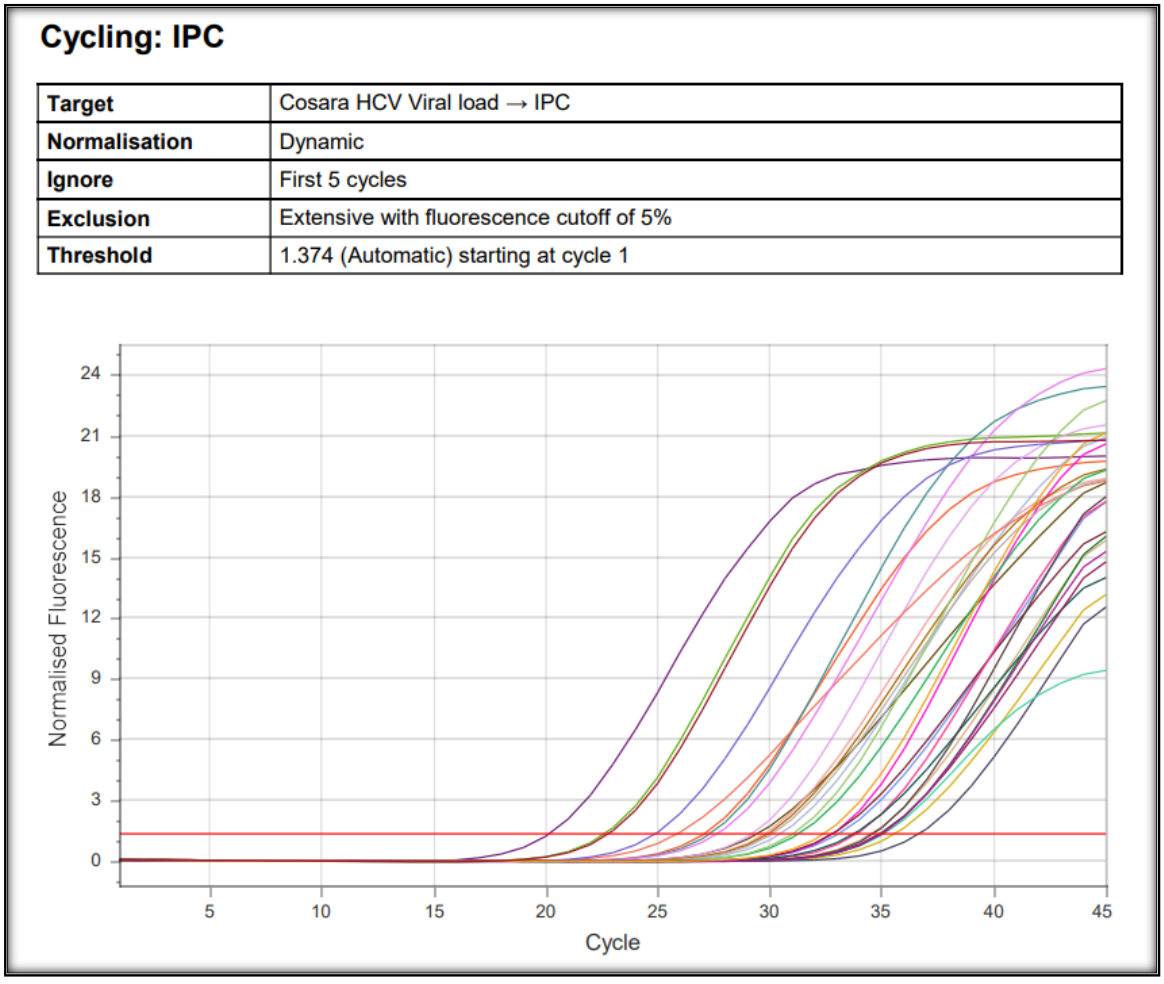


Figure13: Amplification graph of HCV viral load internal positive control (IPC)

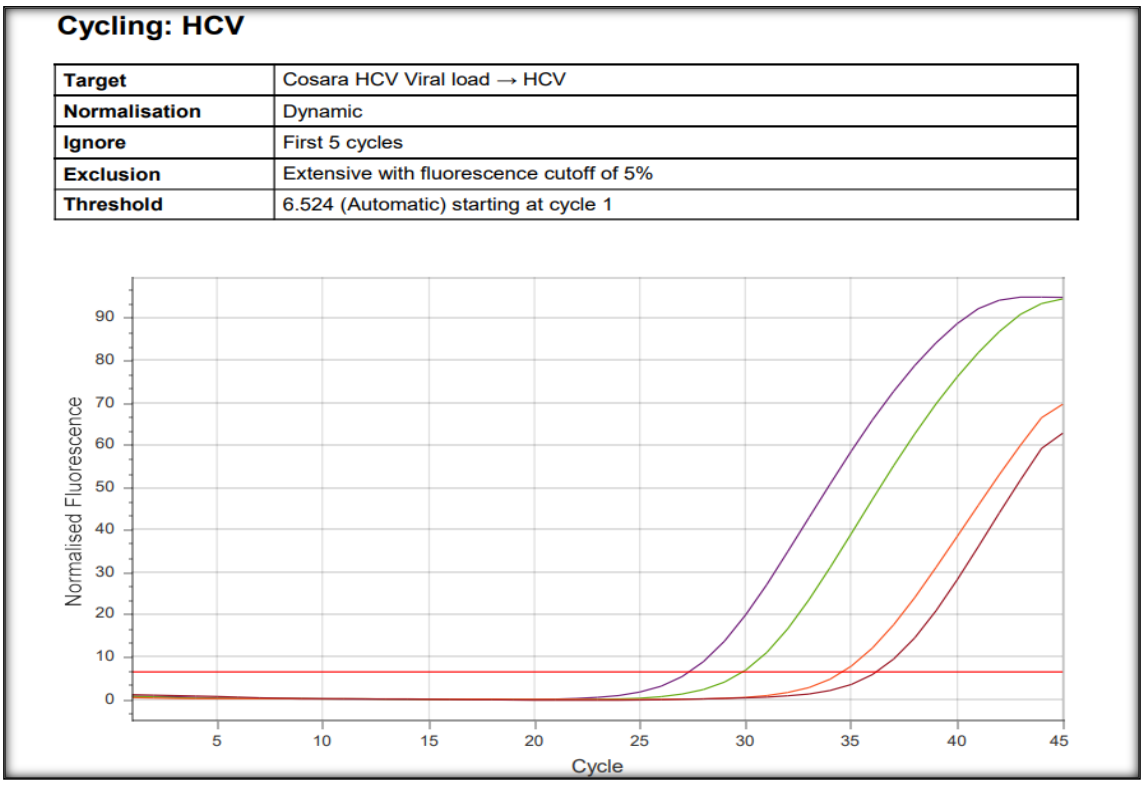


Figure 14: Amplification graph of HCV viral load standard

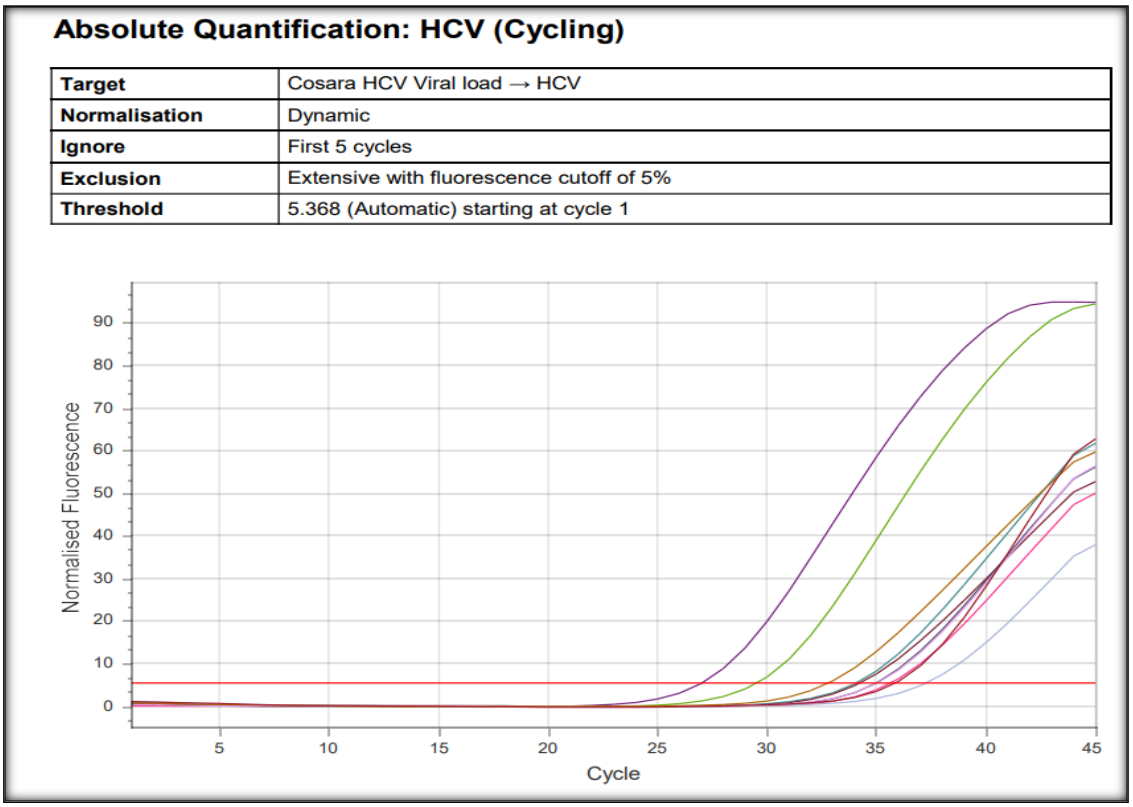


Figure 15: Amplification graph of HCV viral load of patients

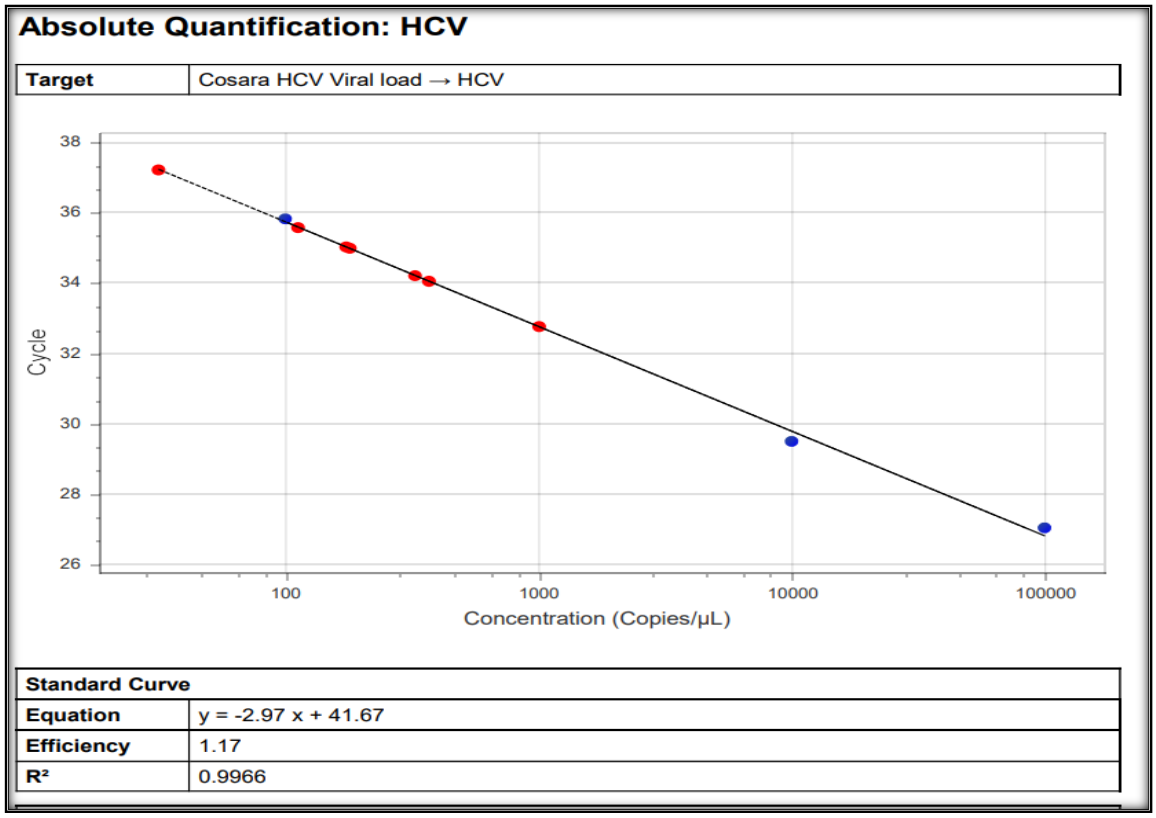


Figure 16: Quantitative plot of HCV viral load copies

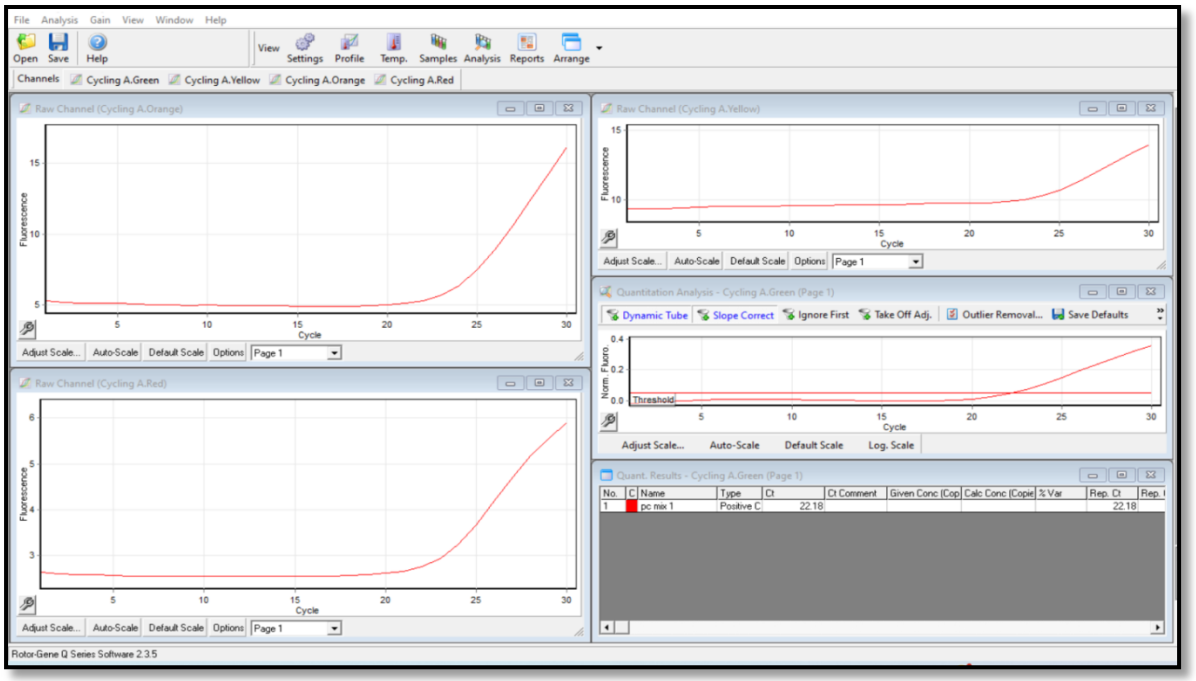


Figure 17: Amplification graph represents the HCV genotypes internal positive control with different channels.

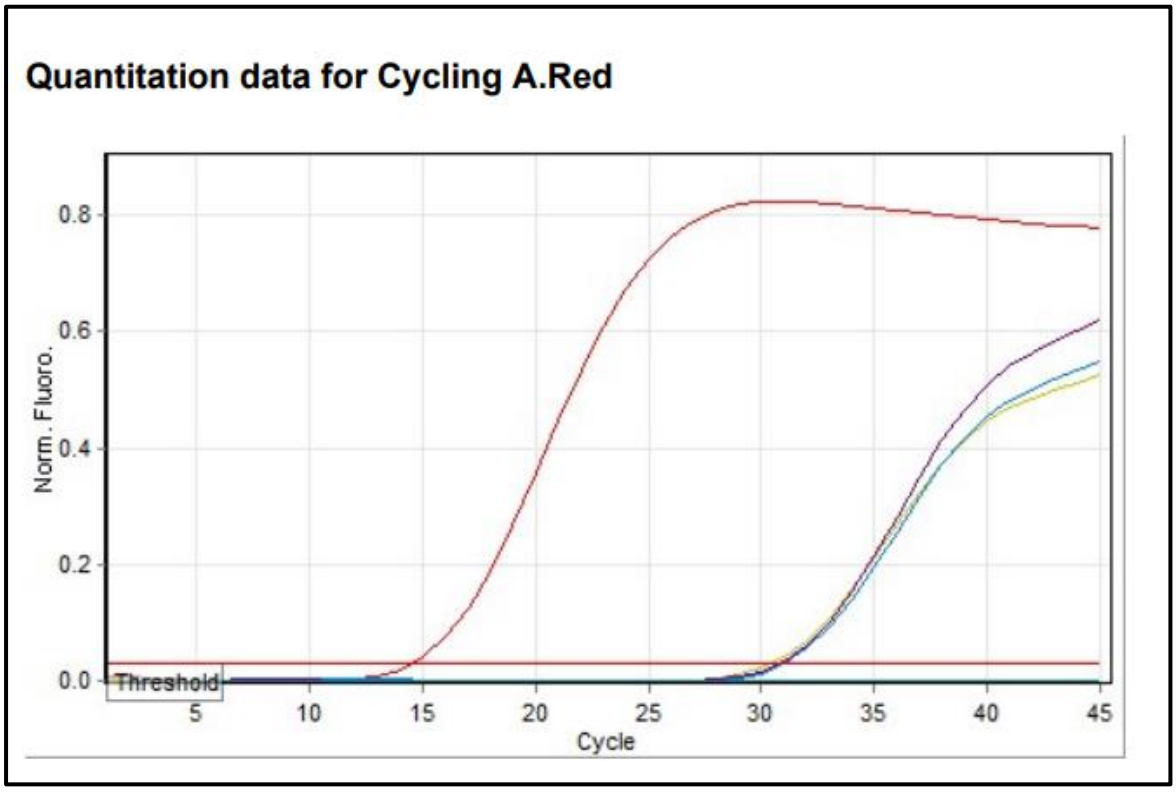
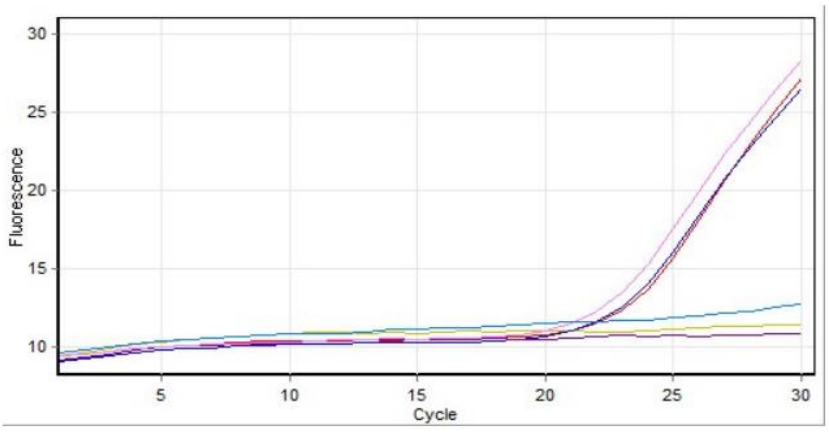


Figure 18: Graph showing the raw and quantitative date of genotype 1 in channel red.

Profile

Cycle	Cycle Point
Hold	Hold @ 95°C, 3min 0s
Cycling (30 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 60°C, 60s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7], [Yellow][2][2])

Raw Data For Cycling A.Green



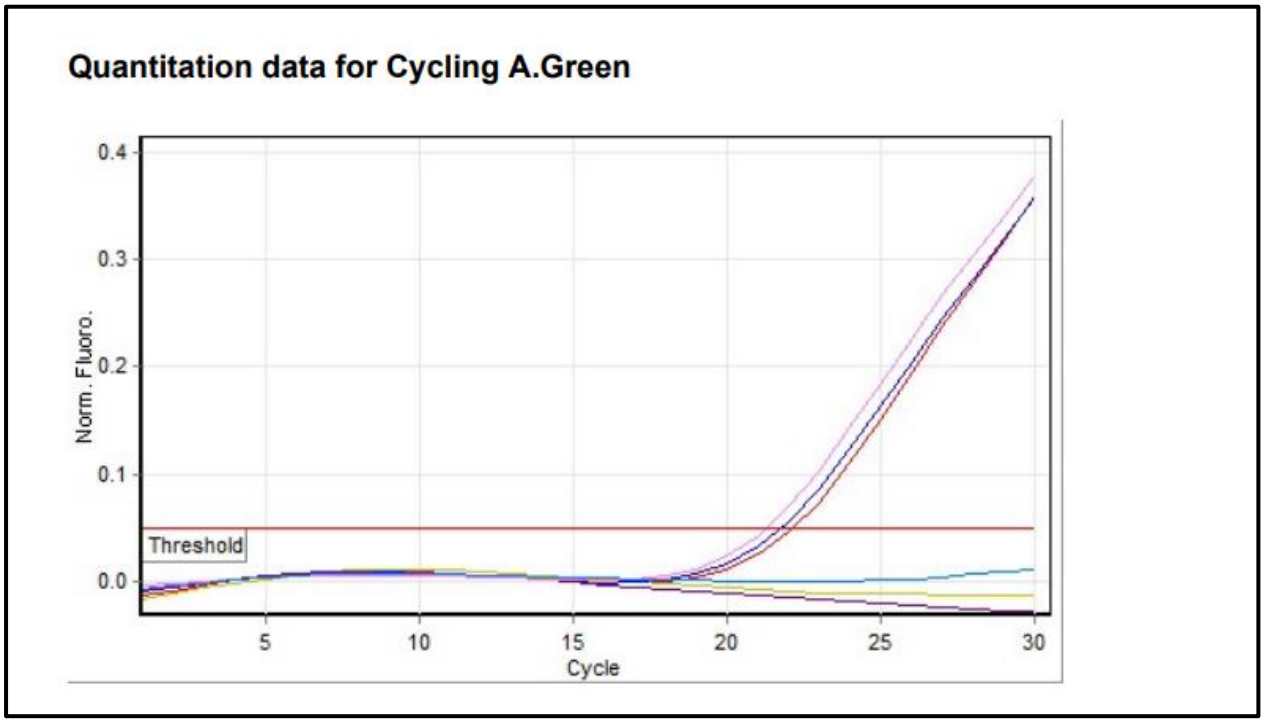
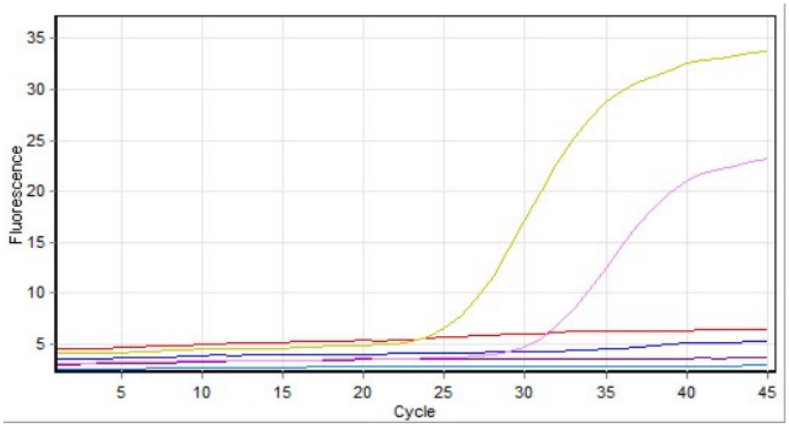


Figure 19: Graph showing the raw and quantitative date of genotype 2 in channel green.

Profile

Cycle	Cycle Point
Hold 1	Hold @ 50°C, 15min 0s
Hold 2	Hold @ 95°C, 10min 0s
Cycling (45 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 55°C, 20s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7], [Yellow][2][2])
	Step 3: Hold @ 72°C, 15s

Raw Data For Cycling A.Orange



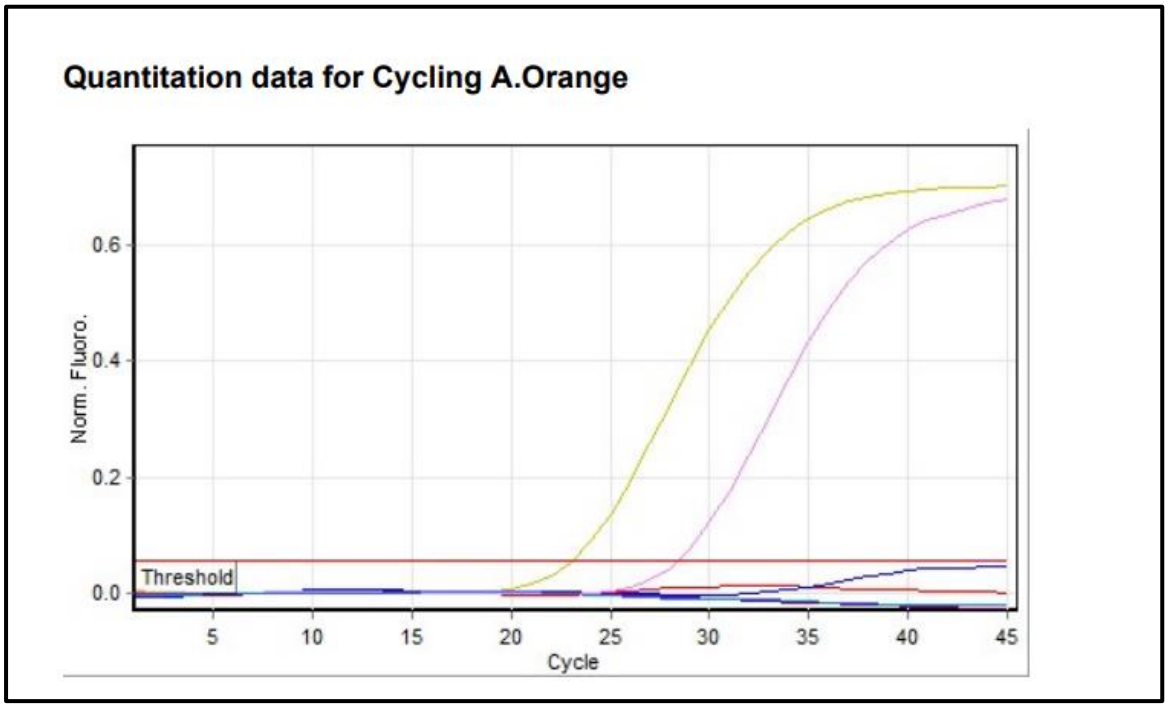
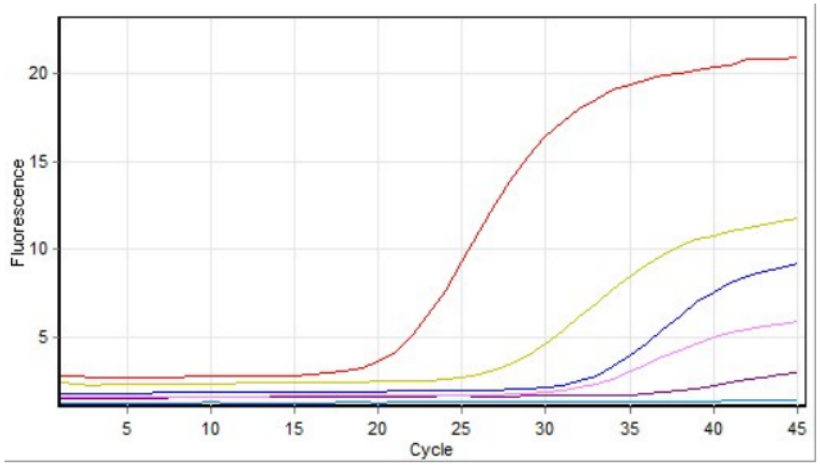


Figure 20: Graph showing the raw and quantitative date of genotype 3 in channel orange.

Profile

Cycle	Cycle Point
Hold 1	Hold @ 50°C, 15min 0s
Hold 2	Hold @ 95°C, 10min 0s
Cycling (45 repeats)	Step 1: Hold @ 95°C, 15s
	Step 2: Hold @ 55°C, 20s, acquiring to Cycling A([Green][1][1],[Orange][3][3],[Red][4][7],[Yellow][2][2])
	Step 3: Hold @ 72°C, 15s

Raw Data For Cycling A.Yellow



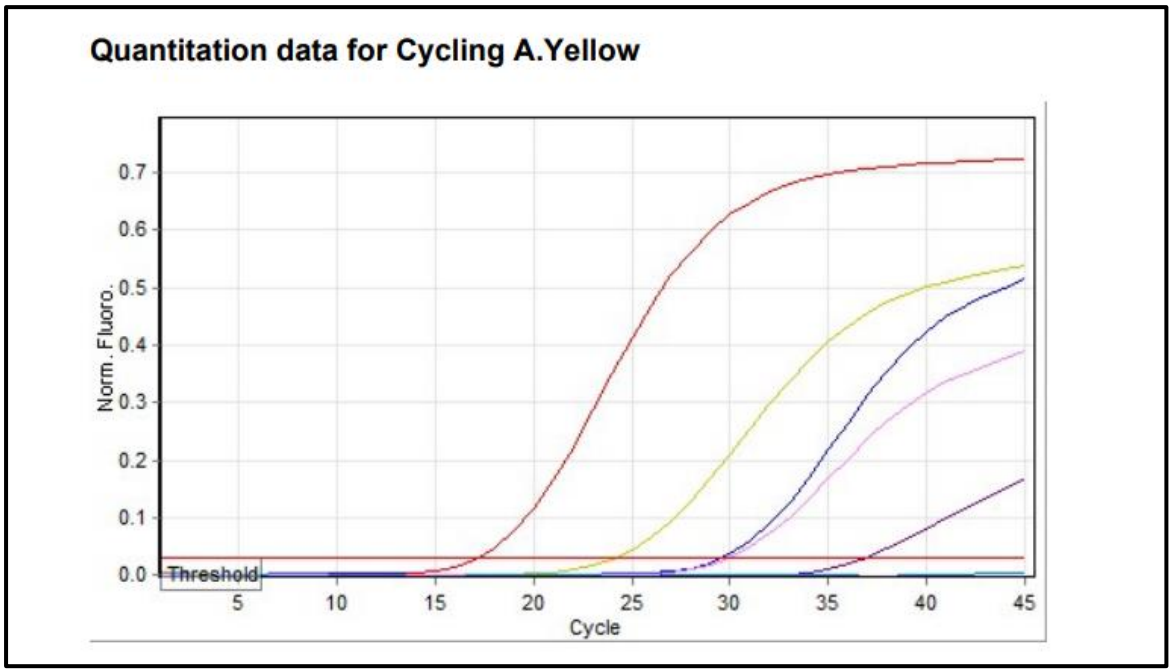


Figure 21: Graph showing the raw and quantitative date of genotype 4 in channel yellow.

Molecular characterization of HCV genotypes in study participants

qPCR was used in order to characterize the HCV genotypes in Kolar population, HCV RNA of all 66 samples with active RNA detected were analysed. In this study HCV genotype 3(50%) was found to be more prevalent followed by genotype 2(16.6%), 1(15.1%), 4(13.6%) and mix (4.5%) Figure: 22.

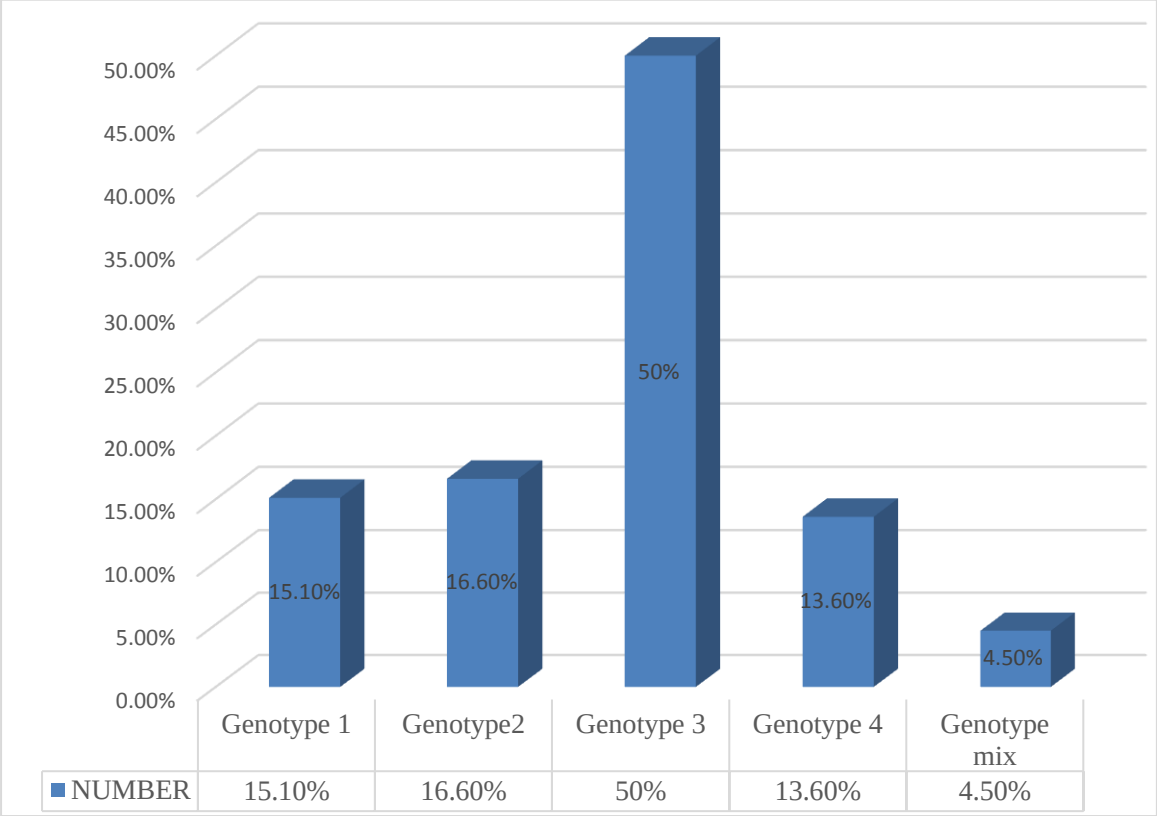


Figure 22: Distribution of HCV genotypes.

Correlation of HCV RNA levels with genotype 1, 2, 3 4, and mix.

The table shows the association of HCV RNA levels with genotypes 1, 2, 3, 4, and the genotype mix (Tables 9, 10, 11, 12, and 13). There was a negative correlation between HCV RNA levels and genotype 1 (p value=0.178). A positive correlation was observed between HCV RNA levels and genotype 2,3,4, and genotype mix (p value= <0.05).

Table 9: Correlation between HCV RNA levels and genotype 1 of the hepatitis C infected patients

Clinical details	Total No. of patients n = 124	HCV RNA +ve n= 66	Genotype 1	Pearson correlation coefficient	P value
Fever	7	0	0	0.381	0.178
Jaundice	4	0	0		
Abdominal pain	0	0	0		
Acute liver disease	15	9	1		
Chronic liver disease	9	9	1		
Cirrhosis	11	6	1		
Cholelithiasis	10	4	1		
Blood transfusion	5	1	0		
Surgery	10	5	2		
Metabolic acidosis	14	1	1		
Fatty liver	15	9	2		
Dialysis	8	7	1		
Drug abuse	0	0	0		
others	16	15	0		

No statistically significant correlation between HCV RNA + and Genotype 1 with (p value=0.178 & r= 0.381)

Table:10. Correlation between HCV RNA levels and genotype 2 of the hepatitis C infected patients

Clinical details	Total No. of patients n = 124	HCV RNA +ve n= 66	Genotype 2	Pearson correlation coefficient	P value
Fever	7	0	0	0.929	0.000
Jaundice	4	0	0		
Abdominal pain	0	0	0		
Acute liver disease	15	9	2		
Chronic liver disease	9	9	2		
Cirrhosis	11	6	1		
Cholelithiasis	10	4	1		
Blood transfusion	5	1	0		
Surgery	10	5	0		
Metabolic acidosis	14	1	0		
Fatty liver	15	9	1		
Dialysis	8	7	1		
Drug abuse	0	0	0		
others	16	15	3		
Total	124	66	11		

Highly statistically significant correlation between HCV RNA+ and genotype 2 (p value=0.00 & r=0.929)

Table: 11 Correlation between HCV RNA levels and genotype 3 of the hepatitis C infected patients.

Clinical details	Total No. of patients n = 124	HCV RNA +ve n= 66	Genotype 3	Pearson correlation coefficient	P value
Fever	7	0	0	0.972	0.00
Jaundice	4	0	0		
Abdominal pain	0	0	0		
Acute liver disease	15	9	4		
Chronic liver disease	9	9	5		
Cirrhosis	11	6	4		
Cholelithiasis	10	4	1		
Blood transfusion	5	1	1		
Surgery	10	5	3		
Metabolic acidosis	14	1	0		
Fatty liver	15	9	5		
Dialysis	8	7	3		
Drug abuse	0	0	0		
others	16	15	7		
Total	124	66	33		

Highly statistically significant correlation between HCV RNA+ and Genotype3(p value<0.05 & r=0.972)

Table:12 Correlation between HCV RNA levels and genotype 4 of the hepatitis C infected patients

Clinical details	Total No. of patients n = 124	HCV RNA +ve n= 66	Genotype 4	Pearson correlation coefficient	P value
Fever	7	0	0	0.842	0.00
Jaundice	4	0	0		
Abdominal pain	0	0	0		
Acute liver disease	15	9	1		
Chronic liver disease	9	9	1		
Cirrhosis	11	6	0		
Cholelithiasis	10	4	1		
Blood transfusion	5	1	0		
Surgery	10	5	0		
Metabolic acidosis	14	1	0		
Fatty liver	15	9	1		
Dialysis	8	7	2		
Drug abuse	0	0	0		
others	16	15	3		

Highly statistically significant correlation between HCV RNA+ and Genotype 4(p value<0.05 & r=0.842)

Table:13 Correlation between HCV RNA levels and genotype mix of the hepatitis C infected patients

Clinical details	Total No. of patients n = 124	HCV RNA +ve n= 66	Genotype mix	Pearson correlation coefficient	P value
Fever	7	0	0	0.706	0.005
Jaundice	4	0	0		
Abdominal pain	0	0	0		
Acute liver disease	15	9	1		
Chronic liver disease	9	9	0		
Cirrhosis	11	6	0		
Cholelithiasis	10	4	0		
Blood transfusion	5	1	0		
Surgery	10	5	0		
Metabolic acidosis	14	1	0		
Fatty liver	15	9	0		
Dialysis	8	7	0		
Drug abuse	0	0	0		
others	16	15	2		

Statistically significant correlation between HCV RNA + and Genotype mix with (p value=0.005 & r=0.706)

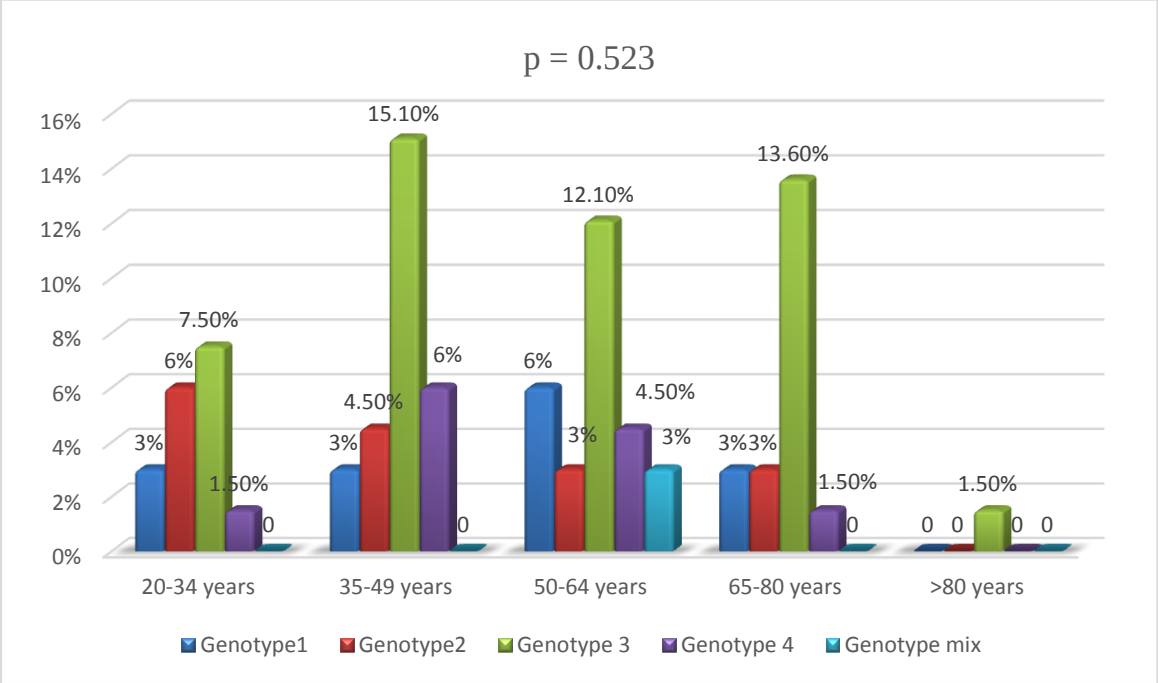


Figure 23: Distribution of HCV genotype in different age groups.

In different age groups the HCV genotypes were distributed as shown in figure: 23 Genotype 3 was found to be more in 35-49 years (15.1%) among all the age groups. There was no statistical difference found in age with genotype distribution (*P*=0.523).

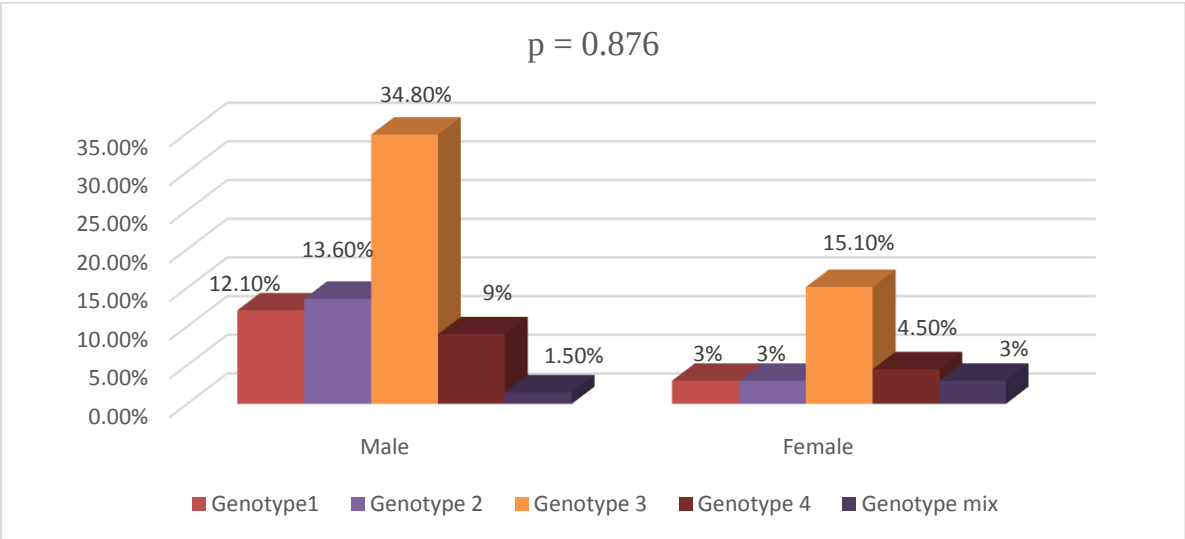


Figure24: Distribution of HCV genotypes among different genders.

Distribution of HCV genotypes with gender is represented in figure 24. The prevalent genotype among males was 3(34.80%), followed by genotype 2(14.2%), 1(11.1%), 4(9.5%) and mixed genotypes (1.5%) whereas in females the predominant genotype among infected female patients was 3(15.1%) followed by genotype 4(5%), 2(3.1%), 1(3.1%) and mixed genotypes (3.1%). There was no significant relationship in gender with genotype distribution (*P*= 0.876).

Comparison of viral load and genotypes.

The median viral load of genotype 1 was 1675200 IU/mL (826500-2295866100), genotype 2 was 12154800 IU/mL (2969550-25652250), genotype 3 was 820800 IU/mL (35100-8542500), genotype 4 was 14013000 IU/mL (79500-16740000) and genotype mix was 518700 IU/mL (142200-5346000). The viral RNA copies were categorized as low viral load < 800,000 IU/mL and high viral load ≥ 800,000 IU/mL (Mishra B Ket al., 2020). There was no significance difference between viral load and genotype s of HCV infected patients (*P*=0.57). Data represented in table 14.

Table 14: Association between HCV viral load and genotypes in study subjects.

Genotype	< 800000 IU/mL	> 800000IU/mL	*p*-value
Genotype 1	3(4.7%)	7(10.6%)	0.57
Genotype 2	3(4.7%)	8(12.6%)	
Genotype 3	16(24.2%)	17(25.7%)	
Genotype 4	4(6.3%)	5(7.9 %)	
Genotype mix	2(3.1%)	1(1.5%)	
Total	28(42.4%)	38(57.5%)	66(100%)

Objective2: To determine the association of IL28B rs12979860 with HCV infection

Genotype and allele frequency of SNPrs.12979860 IL28B

Table 9 displays the findings of IL28B testing on HCV patients and healthy controls. The frequency of the dominant homozygous advantageous GG allele at rs12979860 reported in 52.4% of cases and 70% of controls, while the frequency of the unfavourable AA allele was observed in 28.2% of cases and 8% of controls. The dominant allele G for IL8B rs12979860 is found in 154 cases and 197 controls, while the risk allele is found in 65 cases and 46 controls. When comparing HCV-infected patients and healthy controls, there was no significant

difference in IL 28 B genotype (p = 0.001). Infection with hepatitis C virus is linked to the IL28B rs12979860 gene.

Table 15: IL28B rs12979860 allele and the genotype with HCV and controls.

Gene	Genotype/ Allele	Cases (n= 124)	Controls (n=124)	*P*-value	Odd ratio
IL28B (rs12979860)	GG	65(52.4%)	83(66.9%)	<0.001	-
	GA	24(19.3%)	31(25%)		
	AA	35(28.2%)	10(8%)		
	G	154	197	<0.0001	2.4 (1.6-3.5)
	A	94	51		

Evaluate the association of genetic models of IL282B SNP rs12979860 with HCV.

Different genetic models were used to examine the correlation between IL28BSNP rs12979860 and the risk of contracting hepatitis C virus (HCV), with the results showing the strongest correlation in the recessive model (AA + GA vs. GG, p = 0.01, OR = 4.5).

Table16: Genetic model of SNPs rs12979860 IL28B with HCV and healthy control.

Model	Genotype	*P*-value*	Odds ratio
Dominant	AA + GA vs GG	<0.0001	1.8
Recessive	AA vs GA + GG	<0.0001	4.5
Additive	AA> GA>GG	<0.0001	4.4>0.9>1
Multiplicative	A vs G	<0.0001	2.4

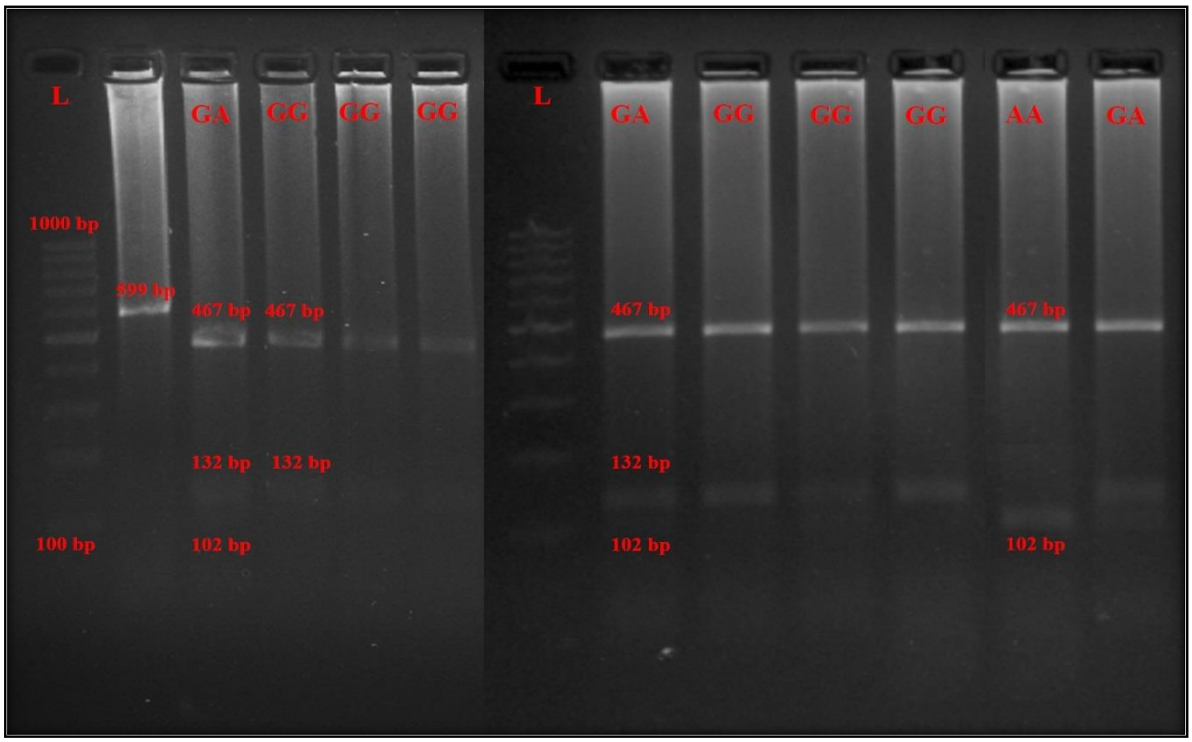


Figure25: PCR-RFLP band pattern of IL28B (rs12979860) in cases. Gel image showing the ladder 1000bp, control 599 bp, wild homozygous genotype GG 467 bp,132bp, heterozygous genotype GA 467 bp,132 bp,102 bp and homozygous genotype 467bp, 102bp. DNA bands on 1% gel was stained with ethidium bromide and run at 110V for 20 min . visualization was done under UV light.

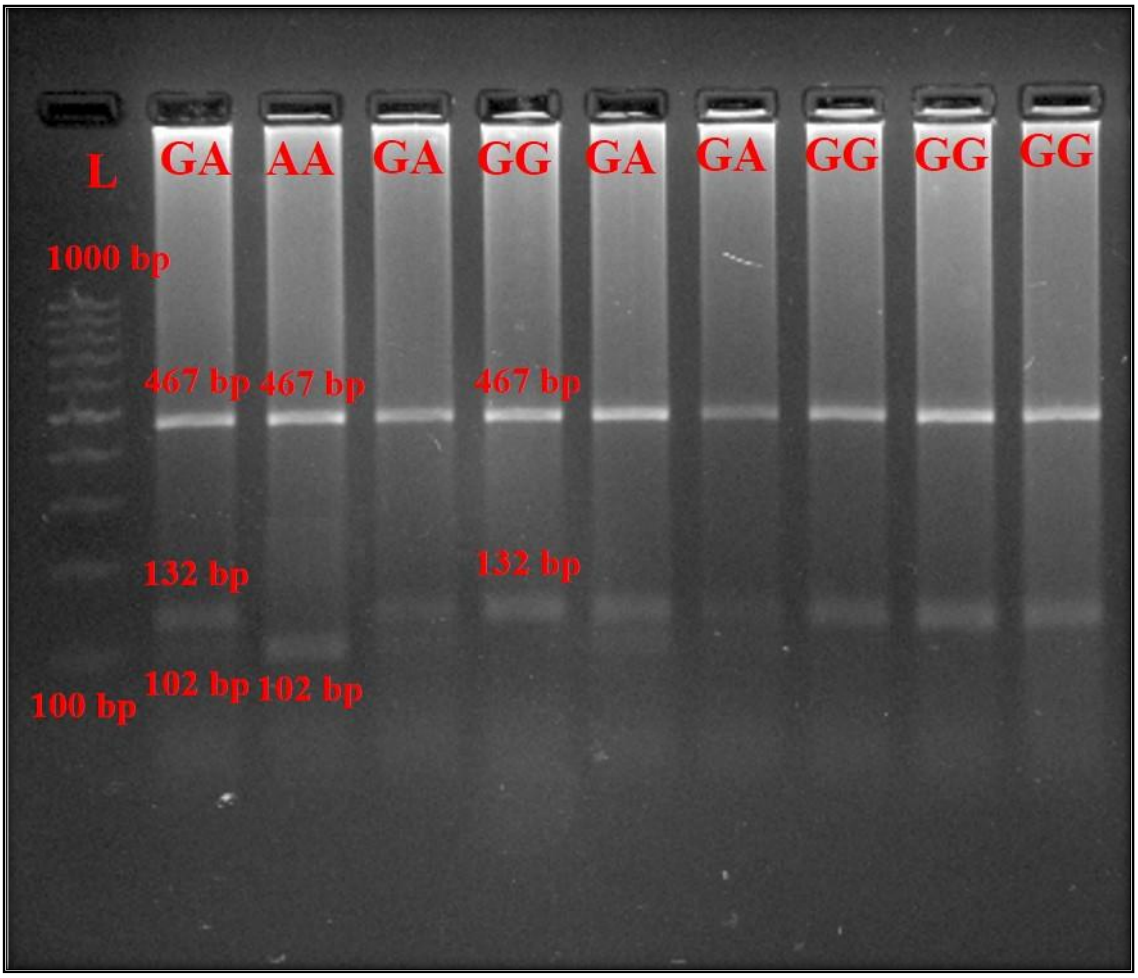


Figure 26: PCR-RFLP band pattern of IL28B (rs12979860) in controls. Gel image showing the ladder 1000bp, control 599 bp, wild homozygous genotype GG 467 bp,132bp, heterozygous genotype GA 467 bp,132 bp,102 bp and homozygous genotype AA 467bp, 102bp. DNA bands on 1% gel was stained with ethidium bromide and run at 110V for 20 min. visualization was done under UV light.

Effect of Viral load on IL28 in Hepatitis C infected patients

All individuals underwent PCR-RFLP genotyping for IL28B.rs12979860 to determine the effect of IL28B on HCV virus load. Since the data did not follow a

normal distribution, we only report the median and interquartile range (IQR). Cases with the GG genotype of IL28B.rs 12979860 had HCV viral loads on average (SEM) of 1.06 x 4.38 x 10^5 IU/mL, while those with the GA genotype had HCV viral loads on average (SEM) of 9.96 x 7.25 x 10^5 IU/mL. Higher viral loads were seen in individuals with the unfavourable 'AA' genotype and lower loads in those with the more common 'GG' genotype. HCV cases having a GG, GA, or AA genotype at the IL28B rs12970860 locus had median viral loads of 189,600, 228,400, and 25,652,250 IU/ML, respectively. Patients infected with HCV had IQRs of 561600, 46912500, and 56802075 IU/mL. Among the 66 HCV-positive individuals, the GG, GA, and AA genotypes for IL28B.rs 12979860 were found to be 47.6%, 17.4%, and 35.0%, respectively. The presence of a high viral load was linked to the presence of the unfavourable genotype AA. Figure 27 also displays a statistically significant (p0.0001) correlation between IL28b and viral load.

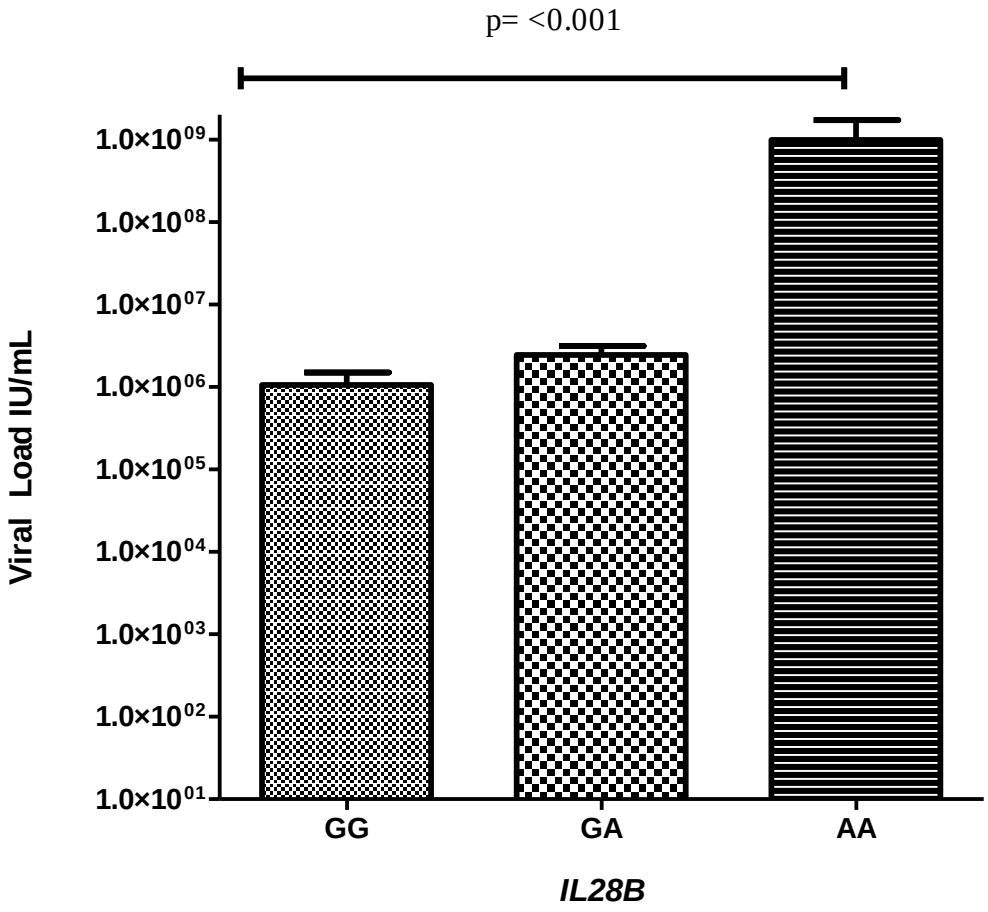


Figure 27: Effect of HCV viral load on SNPS rs12979860 IL28B.

Objective3: To determine the association of TLR2 -196 to -174 del/ins polymorphisms with Hepatitis C infected patients.

TLR2 -196 to -174 del/ins genotype and allele frequency in cases and control.

The genotype frequencies of TLR2 ins/del (-196 to-174) polymorphism in HCV patients and control group. Carrier of wild type TLR2 ins/ins genotype represented (58%) and (63%) in control group. Data indicated there were no statistical difference between the cases and the control group (p = 0.61) (dominant TLR2ins/ins vs TLR2ins/del+ TLR2del/Del, OR= 1.2 (0.8-1.8).

Table 17: TLR2 -196 to -174 del/ins genotype and allele frequency in cases and control.

Genotype	**Genotype /allele**	**Cases (*n* = 124)**	**Control (*n* = 124)**	***p*-value**	**Odds ratio**
TLR2 -196 to -174 del/ins	**Ins**	72(58%)	79(63.7%)	0.16	-
	Ins/ Del	40(32.2%)	36(29%)		
	Del	12(9.6%)	9(7.2%)		
	Ins	184	194	0.14	1.24 (0.8-1.8)
	Del	12	9		

Table 18: Genetic model of TLR2 -196 to -174 del/ins polymorphisms with cases and control.

Model	Genotype	*P*-value*	Odd ratio
Dominant	Del + Ins/Del vs Ins	0.18	1.267(0.75-2.11)
Recessive	Del vs Ins/Del+ Ins	0.25	1.367(0.5-3.49)
Additive	Del > Ins/Del>Ins	-	1>0.8>0.6
Multiplicative	Ins vs. Del	0.1472	1.24 (0.8-1.8)

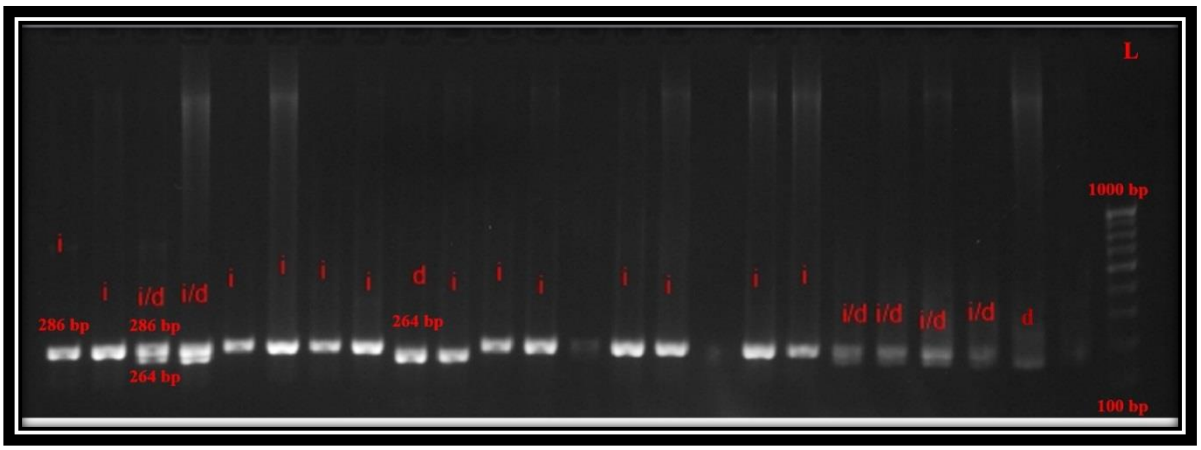


Figure:28 PCR- gel electrophoresis band pattern of TLR2 (-196 to -174 del/ins) in cases. Gel image showing the ladder 1000bp, wild homozygous genotype Ins/ins 286 bp, heterozygous genotype Ins/ del 286 bp,264 bp and homozygous genotype deletion 264 bp. DNA bands on 1% gel was stained with ethidium bromide and run at 110V for 20 min. visualization was done under UV light.

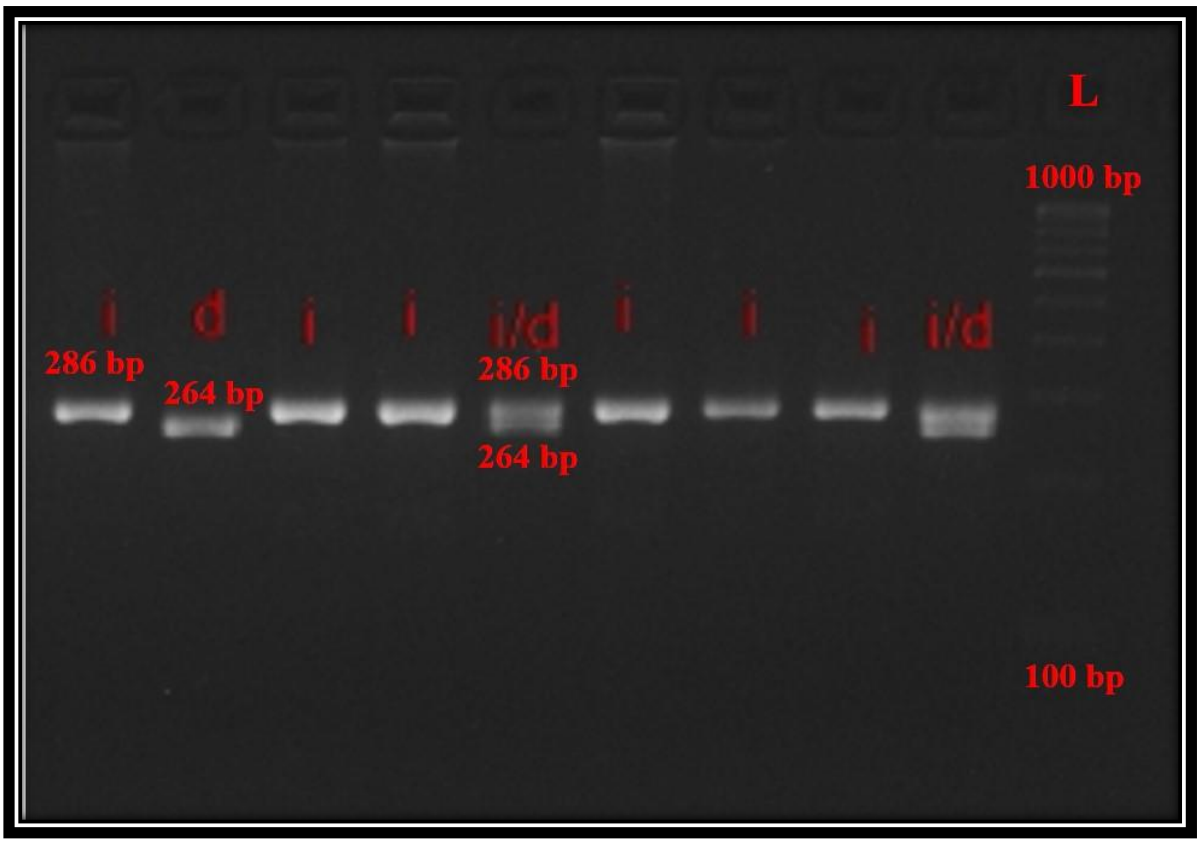


Figure:29 PCR-gel electrophoresis band pattern of TLR2 (-196 to -174 del/ins) in controls. Gel image showing the ladder 1000bp, wild homozygous genotype Ins/ins 286 bp, heterozygous genotype Ins/ del 286 bp,264 bp and homozygous genotype deletion 264 bp. DNA bands on 1% gel was stained with ethidium bromide and run at 110V for 20 min. visualization was done under UV light.

CHAPTER VII

DISCUSSION

A major public health issue, HCV has been established as a leading cause of liver disease. The spread of HCV infection, also known as blood-borne non-A non-B infection, is a major global health issue (Testino G et al., 2013). Naturally curing HCV occurs in 20-45% of infections, depending on immunological features, typically within the first 6 months of exposure. (Bulut ME et al., 2021).

It is important to understand the genotypes of the hepatitis C virus (HCV) while designing the therapeutic approaches. Quantitative HCV RNA testing yields prognostic information helpful for evaluating the success of antiviral treatment. (Ournasseir Meet al., 2019). The nucleotide sequence of HCV displays substantial diversity. Some parts of the viral genome, like the 5' untranslated region (UTR), are remarkably conserved, whereas other parts, like the envelope region's HVR, show up to 51% sequence diversity. The current hierarchical classification of six genotypes and over a hundred subgroups has been justified by genotyping analysis **(**Cooreman MP et al., 1996). So, the purpose of the study was to define the HCV genotypes and the polymorphism of SNPs *IL28B* and *TLR2* gene in HCV infection, in the Kolar population.

Objective 1: To determine the HCV prevalent genotype.

In order to determine the status of HCV infection among the study subjects, viral load measurement and qPCR genotyping were performed on all antibody-positive participants.

Active HCV RNA infection was found to be 53.2% (66/124) in the current investigation. According to studies conducted in Turkey and North China rate of active infection was 33.17% and 42.9%. Multiple studies have demonstrated the feasibility of using HCV RNA testing as a screening tool. (Alacam S et al., 2022; Li Y et al, 2020; Kyuregyan K.K et al., 2019).

Fifty percent of participants in this study had the genotype 3, followed by 16 percent with genotype 2, 15 percent with genotype 1, 16 percent with genotype 4, 13 percent with a mixed genotype, and 4 percent with genotype 1. A study conducted by Christdas. J et al in 2013 observed that Genotype 3 was found to be the most common (63.85%), followed by genotype 1 (25.72%), genotype 4 (7.5%), genotype 6 (2.7%), and genotype 2 (0.002%).

Another study conducted by Basharkhah. S et al in 2019 depict that Genotype 3 (46.6%), followed by genotype 1 (42.6%), genotype 2 (5.2%), and a mixed genotype (1.2%). A similar study done by Hadinedoushan H et al. in 2014 found that a majority of HCV infections were of genotype 3 (50.3%), whereas 2.6% were of a mixed genotype, and 1.6% were of genotype 2.

Genotypic variation among HCV carriers is a result of the inherent diversity of the HCV genome. Contrary to our findings, Roy P et al.2011 discovered that genotype 1a was the most common (54.1% of the population), followed by 1b (43.2%) and 3a (2.7%). The majority of people have the first genotype (81.3%), followed by those with the third (8.8%), the second (3.4%), the fourth (2.9%), the fifth (0.5%), and those with a different genotype (2.9%). Audu RA et al. in 2015 found that only

63.2% of Nigerians were of genotype 1, while 16.8% were of genotype 4, 10.5% of genotype 3, 7.4% of genotype 2, and 2.1% were of mixed genotypes.

HCV primarily spreads parenteral route, and common sources of infection includes injections, drug addiction, needle stick mishaps, and blood transfusion and its byproducts transfusion. In our study, majority of the HCV infections were with patient admitted with trauma and accidental cases, and angioplasty, acute liver disease, chronic liver diseases, fatty liver, cirrhosis. These risk factors showed a significant correlation with the genotype 2,3,4 and genotype mix (p= <0.005) whereas no significant correlation with the risk factors was observed with genotype 1(p= 0.178). A study conducted by the Chakravarti. A., et al in 2011 found that the predominant risk factors associated with HCV infection were surgery, blood transfusion, and dental procedure followed by tattooing and I.V. drug use. In a study done by Balew M et al in 2014, in Ethiopia where a history of hospitalization, tooth extraction, and blood transfusion was identified as major risk factors for HCV infection. In contrast, a study conducted by Alemayehu et al in 2011, there were no significant risk factors associated with HCV infection.

Our study also analysed the correlation between HCV RNA-positive patients with different HCV genotypes. There was no correlation observed between HCV RNA-positive and genotype 1(p= 0.178). HCV Genotype 2,3,4, and mix showed a significant correlation with HCV RNA positive(p= <0.005). Chakravarti. A et al in 2011 found that Genotype 1 was associated with a significant (P= 0.0001) HCV RNA positive as compared to Genotypes 3 and 2. Xiang Y et al 2019 observed

positive correlation between HCV RNA and genotype 1 and also with genotype 3 (P = 0.04).

In the present study, the distribution of genotypes with age was also observed. Our results found that the genotype 3 was more predominant in the age group of 35-49 years (15.1%) among different category of age groups. The results showed that there was no statistical significance between the HCV genotypes with age (p = 0.523). similar study conducted by Ali et al., in 2011 also that in young age patients genotype 3a more predominant (45.8%). Study contradicts with Nafees et al., 2007, who reported that highest infection was noted in the age group 40-49 years that was 35.6%. Another study conducted in Eastern India in year 2019 by Roy et al, also reported that 60% of the patients were in the age group 51-60 years. Our study also found the gender was also determined. The results showed there was no statistical significance between the HCV genotypes with gender (p =0.876). A study conducted by the Idrees M in 2008 also found there was no statistically difference between the gender.

Here, we find no statistically significant association (P=0.57) between HCV genotypes and viral load. Although the difference between genotype 1 and 2 patients and genotype 3 patients was not statistically significant (p>0.5), it was reported in research by Bhattacharjee D et al in 2015. In Pakistan, Ali. A. et al in 2011. found that patients infected with HCV of genotype 3 had a significantly higher viral load than those infected with any other genotype. Patients infected with genotype 4 showed a higher viral load than those infected with genotypes 1,

2, or 3, contrary to our findings. There was a substantial association between HCV genotypes and viral load, as demonstrated by the research conducted by Riaz.S et al. in 2016 on the Sindh population. Patients infected with genotype 3 exhibited a significantly higher viral load, as demonstrated by the work of Mishra BK et al. in2020.

The most widely distributed genotypes are types 1, 2, and 3, whereas other genotypes are restricted to specific regions. Genotyping for HCV can shed light on questions about the spread of the pandemic as well as the variability of the virus's genome (Lazo M et al., 2008 Liu J et al., 2005). Genotypes of HCV are useful epidemiological markers and can influence the efficiency of diagnostic tests for the virus. Accurately identifying and characterizing HCV genotypes and subtypes is crucial to the development of an HCV vaccine (Zein NN et al., 2000).

Objective 2: To determine the association of SNPs *IL28B (rs12979860)* with HCV infection

The present investigation found that the IL28 B rs12979860 SNP was associated with both HCV-infected patients and healthy controls. When compared to healthy controls, the frequency of the IL28 B homozygous favorable genotype GG was 83(70%), heterozygous 31(52%), and homozygous unfavorable allele AA 10(8%), which was statistically significant (P= 0.001). For SNP rs12979860, the favorable allele G frequency was 154 and the unfavorable allele A frequency was 94 in cases, whereas in controls, the frequency of the favorable allele G was 197 and the

unfavorable allele A was 51, showing a statistically significant difference (P= 0.001) with an odd ratio of 2.4. Study results from Sivaprasad S et al. in 2012 indicated that the CC genotype was significantly more common than the CT (34.09%) and TT (6.81%t) genotypes. The frequency of the major allele C is 0.762, whereas that of the minor allele T is 0.238. High levels of the 'CC' genotype of the IL28B SNP, rs12979860C/T, indicating that HCV patients will have a greater response to conventional anti-HCV treatment. In IL 28B rs12979860, Pasha HF et al. in 2013 found that 30.7% of people had the favorable homozygous genotype CC, 49.5% had the heterozygous genotype CT, and 19.8% had the homozygous unfavorable genotype TT. T allele carrier status was significantly associated with HCV infection (P 0.001). That IL28 B is linked to HCV infection, as found here, was also found in the aforementioned study.

The frequency of IL28B rs12979860 genotype GG, GA, and AA among the 66 HCV infected patients was 47.6%, 17.4%, and 35%, respectively, in the present study, demonstrating the impact of HCV viral load on the IL28 B genotype. There was a statistically significant (P = 0.001) difference between those with the unfavorable AA allele and those without it when it came to viral load. Higher than average quantities of HCV RNA were identified in individuals with the IL28 B rs 12979860/rs8099917 TT/CC genotypes, according to research by Boglione L et al. in 2015. Grebely et al 2014 reported a significant finding that IL28B genotype in HCV RNA levels can affect the liver inflammation in HCV -infected patients. While a study by Abdelwahab SF et al. in 2015 found that people with the

"favorable" CC genotype had a lower viral burden than those with the "unfavorable" TT genotype, this difference was not statistically significant.

Objective3: To determine the association of SNPs *TLR2 (-196 to -174 del/ins)* with HCV infection

TLR2 -196 to -174 del/ins was shown to be associated with both HCV-infected patients and healthy controls in the present investigation. TLR2 was more common in those with a homozygous favorable genotype insertion (58%), a heterozygous insertion /deletion (32.2%), and a homozygous unfavorable allele deletion (9.6%) compared to healthy controls (63%), although the difference was not significant (P= 0.16). The frequency of the protective allele of SNP TLR2 was 184, whereas that of the risk allele was just 12. There was no significant difference between the frequency of the favorable allele insertion (194) and the frequency of the unfavorable allele deletion (9), with an odd ratio of 1.24 (P= 0.14). No association between the TLR2 SNP and HCV infection was found. Nischalke HD et al.2012 highlighted a strong correlation between HCV infection and the TLR2. Research by Neamatallah M et al. in 2020 conducted a study that minor allele of TLR 2 was associated with the outcome of HCV infection. This is the first study of its kind in India, as far as we are aware.

SUMMARY AND CONCLUSION

In the present investigation, we compared HCV-infected individuals and healthy controls for their HCV genotype and for their IL28B and TLR2 polymorphism associations. Results from this study improve our understanding of the HCV genotype distribution among HCV patients in Kolar, Karnataka. Genotype 3 was shown to be the most prevalent in this study, with genotype 1 coming in second. Neither viral load nor HCV genotypes were shown to be significantly related to one another. The prevalence of HCV infection among young people is a big worry, as shown by our research. In contrast to the TLR2 -196 to -174 del/ins polymorphisms, the IL28Brs12979860 SNP was found to be related with HCV infection. In the future, IL28 B has the potential to be employed as a therapeutic target and to contribute to vaccine development.

NEW KNOWLEDGE GENERATED

To the best of our knowledge, this is the first study to analyse HCV genotypes in the Kolar area. This is the first study in India, related to TLR2 -196 to -174 del/ins polymorphism. This new sight adds a beneficial effect of IL28 B and TLR2 polymorphism in vaccine development and therapeutic potential.

LIMITATION OF THE STUDY

To fully grasp the scope of the problem in the Kolar area, a large number of sample is required. Selected HCV genotypes were identified. The subtypes of the HCV genotype were not identified. Single nucleotide polymorphisms (SNPs) were the only variants in the IL28 B and TLR2 genes considered in this research.

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LIST OF PRESENTATIONS AND PUBLICATIONS

Presentations:

1. Devinder Kaur, Prabhakar K. Distribution of genotypes in Hepatitis C infected patients in tertiary care hospital. Poster presentation at international webinar on 2 Aug to 3 August 2021.
2. Devinder Kaur, Prabhakar K.the distribution of genotype IL28 B gene polymorphism in hepatitis C infected patients. Poster presentation at webinar on global and genome evalution on 27Aug to 28 August 2021.
3. Devinder Kaur, Prabhakar K. Interlukin 28B and TLR2 polymorphism in HCV infected patients. Oral presentation at ACP on 18 Nov to 20 November 2022.

Publication:

1. Kaur D, Prabhakar K, Das S. Distribution of HCV genotypes and HCV RNA viral load in hepatitis infected patients of Kolar region, Karnataka, India. Bioinformation. 2022;18(4):387-91.

Conference Mind

CERTIFICATE OF RECOGNITION

Devinder Kaur

(Medical Acupuncture And Pain Management Clinic,Brasil)

For sharing her valuable knowledge at the International Webinar on Microbiology, during August 02-03, 2021 with her phenomenal and worthy Poster presentation entitled "Distribution of genotyping in hepatitis C infected patients in tertiary care hospital"

https://www.conferencemind.com/

Ranjan Panda
C.E.O,Conference Mind

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GENOME EVOLUTION 2021

CERTIFICATE OF RECOGNITION

Is hereby presented to

Devinder Kaur

(Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka, India)

For sharing her valuable knowledge as Honorable Speaker at Global Webinar on Molecular And Genome Evolution to be held during August 27-28, 2021. with her phenomenal and worthy Poster presentation entitled "The Distribution of Genotype and Allelic Frequency of IL28B Gene Polymorphism in hepatitis C infected patients"

Ranjan Panda
(CEO Conference Mind)


ACP American College of Physicians Leading Internal Medicine, Improving Lives
India Chapter
7th ANNUAL HYBRID MEETING OF THE IM ACP INDIA 2022
18th - 20th November 2022, Hotel Novotel, Visakhapatnam AP, India
Certificate
This is to certify that
Devinder Kaur
has presented Free Paper Session on
INTERLEUKIN 28B AND TOLL LIKE RECEPTOR 2 GENE POLYMORPHISM IN HEPATITIS C INFECTION
7th ANNUAL HYBRID MEETING OF THE IM ACP INDIA 2022
held on 18th - 20th November 2022, at Hotel Novotel, Visakhapatnam AP, India
Dr. Anuj Maheshwari
Governor, ACP India Chapter
Dr. L. Sreenivasa Murthy
Organizing Secretary
Dr. Narsingh Verma
Scientific Chairman
Dr. Anubha Srivastava
Scientific Secretary

Annexure

ANNEXURE – I
CASE HISTORY SHEET
Title: Detection of genotypes and with association of IL28B and toll like receptor 2 polymorphism in Hepatitis C infected patients.

CASE NO.

Patient name:

Age: Sex:

General Id: Occupational status:

Address:

OP.no: IP.no:

Date of admission:

Abdominal pain

Onset	yes /no
Frequency	yes /no
Type	yes /no
Vomiting	yes /no
UGI bleed	yes /no

If yes ……

Number1/2/more Any history of promisenity Jaundice

Prodrome	yes /no
Onset	yes /no
Duration stage of jaundice	yes /no
Pale stool	yes /no
Fever	yes /no
Chills	yes /no
Typhoid fever	yes /no

Any antibiotics
Anorexia yes /no

Nature yes /no

Smoking yes /no

Oliguria yes /no

Diarrhoea yes /no

Bleeding from rectum yes /no

Sleep disturbance yes /no

Frequent infection RTI/GIT/UTI

Allergy skin allergy /other Alcohol consumption yes /no Duration

Frequency yes /no

Drug abuse
Type yes /no

Hepatoxic drugs
Oral contraceptive yes /no

Blood transfusion in past 6 months yes /no
Blood products yes /no

Any other systemic illness TB/DM/HBP/vascular disease /STD/Metabolic

Any history of reaction with injection/surgery yes /no

If yes type if surgery

Laboratory investigation

Liver function Test:

Serological markers
Anti –HCV

Molecular diagnosis

HCV RNA Viral load HCV genotype /subtype

ANNEXURE – II

INFORMED CONSENT FORM

Title of the Project: Detection of genotypes and with association of IL28B and Toll like receptor 2 polymorphism in Hepatitis C infected patients.

I understand that I remain free to withdraw from this study at any time.

I have read or had read to me & understood the purpose of this study & the confidentiality of the information that will be collected & disclosed during the study.

I have had the opportunity to ask my questions regarding the various aspects of this study & my questions have been answered to my satisfaction.

I agree to participate in this study & authorize the collection & disclosure of my personal information as outlined in this consent form.

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Participant's name & signature/thumb impression **Date**

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Name and Signature of the witness **Date**

Signature of the principal investigator

ಅನುಬಂಧ – II

ತಿಳುವಳಿಕೆಯ ಅನುಮೋದನೆ ಪತ್ರ

ಯೋಜನೆಯಶೀರ್ಷಿಕೆ: ಹೆಪಟೈಟಿಸ್ ಸಿ ಸೋಂಕಿತ ರೋಗಿಗಳಲ್ಲಿ ಜಿನೋಟೈಪ್‌ಗಳ ಪತ್ತೆ ಮತ್ತು ಐಎಲ್ 28 ಬಿ ಮತ್ತು ಟೋಲ್ ನಂತಹ ರಿಸೆಪ್ಟರ್ 2 ಪಾಲಿಮಾರ್ಫಿಸಂನೊಂದಿಗಿನ ಸಂಬಂಧ.

ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಿಂದಹಿಂದೆ ಸರಿಯಲು ಮುಕ್ತನಾಗಿರುತ್ತೇನೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಓದಿದ್ದೇನೆಅಥವಾಅದನ್ನುನನಗೆಓದಿದೆ. ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶ ಮತ್ತು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿದ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವ ಮಾಹಿತಿಯ ಗೌಪ್ಯತೆಯನ್ನು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಈ ಅಧ್ಯಯನದ ವಿವಿಧ ಅಂಶಗಳಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶಸಿಕ್ಕಿದೆ ಮತ್ತು ನನ್ನ ತೃಪ್ತಿಗೆ ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಲಾಗಿದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ ಮತ್ತು ಈ ಒಪ್ಪಿಗೆಯರೂಪದಲ್ಲಿ ವಿವರಿಸಿರುವಂತೆ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯ ಸಂಗ್ರಹ ಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವಿಕೆಯನ್ನು ಅಧಿಕೃತಗೊಳಿಸುತ್ತೇನೆ.

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ಭಾಗವಹಿಸುವವರ ಹೆಸರು ಮತ್ತು ಸಹಿ / ಹೆಬ್ಬೆರಳು ಅನಿಸಿಕೆ ದಿನಾಂಕ

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ಸಾಕ್ಷಿಯ ಹೆಸರು ಮತ್ತು ಸಹಿ ದಿನಾಂಕ

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿಯ ಸಹಿ

ANNEXURE - III

PATIENT INFORMATION SHEET

Title: Detection of genotypes and with association of IL28B and toll like receptor 2 polymorphism in Hepatitis C infected patients.

Consent for the interview:

Introduction: My name is:

Hepatitis C virus (HCV) is an emerging infection worldwide and the many people infected with its prevalance increasing every year. Viral hepatitis is a cause for major health care burden in India and is now equated as a threat comparable to the „„big three"" communicable diseases – HIV/AIDS, malaria and tuberculosis. Hepatitis A virus and Hepatitis E virus are predominantly enterically transmitted pathogens and are responsible to cause both sporadic infections and epidemics of acute viral hepatitis. Hepatitis B virus and Hepatitis C virus are predominantly spread via parenteral route and are responsible to cause chronic hepatitis which can lead to grave complications including cirrhosis of liver and hepatocellular carcinoma. Poor blood transfusion methods along with unsafe injection practices are potential sources for the rapid spread of infection.

We would like to collect 3 ml of blood sample from you. This research project and this may or may not directly benefit the patient, ensure some research questions which will help understanding the diseases for better patient care and optimal treatment strategy. All information collected from you will be strictly confidential and will not be disclosed to any outsider except if it is required by the law. The information and the samples collected will be used only for the research. The information will not reveal your identity. There is no compulsion to participate in this study. You will be no way affected if you do not wish to participate in this study. You are required to sign only if you voluntarily agree to participate in this study. Further you are at a liberty to withdrawal at any point of time which will not affect your treatment by the concerned physician in any way. All the analysis of the parameters for the patients will be carried free of cost without making marking burden on the patient. This document will be stored in the safe locker in the Department of Microbiology and a copy given to you for information.

For any clarification you are free to contact the investigator:

Name: Devinder Kaur.

Contact Number: 9056432212

ಅನುಬಂಧ – III

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆ

ಯೋಜನೆಯಶೀರ್ಷಿಕೆ: ಹೆಪಟೈಟಿಸ್ ಸಿ ಸೋಂಕಿತ ರೋಗಿಗಳಲ್ಲಿ ಜಿನೋಟೈಪ್‌ಗಳ ಪತ್ತೆ ಮತ್ತು ಐಎಲ್ 28 ಬಿ ಮತ್ತು ಟೋಲ್ ನಂತಹ ರಿಸೆಪ್ಟರ್ 2 ಪಾಲಿಮಾರ್ಫಿಸಂನೊಂದಿಗಿನ ಸಂಬಂಧ.

ಸಂದರ್ಶನಕ್ಕೆ ಒಪ್ಪಿಗೆ:

ಪರಿಚಯ: ನನ್ನ ಹೆಸರು:

ಹೆಪಟೈಟಿಸ್ ಸಿ ವೈರಸ್ (ಎಚ್‌ಸಿವಿ) ವಿಶ್ವಾದ್ಯಂತ ಉದಯೋನ್ಮುಖ ಸೋಂಕು ಮತ್ತು ಪ್ರತಿ ವರ್ಷವೂ ಅದರ ಹರಡುವಿಕೆಯಿಂದ ಸೋಂಕಿಗೆ ಒಳಗಾದ ಅನೇಕ ಜನರು. ವೈರಲ್ ಹೆಪಟೈಟಿಸ್ ಭಾರತದಲ್ಲಿ ಪ್ರಮುಖ ಆರೋಗ್ಯ ರಕ್ಷಣೆಯ ಹೊರೆಗೆ ಕಾರಣವಾಗಿದೆ ಮತ್ತು ಇದನ್ನು ಈಗ ದೊಡ್ಡ ಮೂರು ಸಂವಹನ ರೋಗಗಳಿಗೆ ಹೋಲಿಸಬಹುದಾದ ಬೆದರಿಕೆಯಾಗಿ ಪರಿಗಣಿಸಲಾಗಿದೆ - ಎಚ್‌ಐವಿ / ಏಡ್ಸ್, ಮಲೇರಿಯಾ ಮತ್ತು ಕ್ಷಯ. ಹೆಪಟೈಟಿಸ್ ಎ ವೈರಸ್ ಮತ್ತು ಹೆಪಟೈಟಿಸ್ ಇ ವೈರಸ್ ಪ್ರಧಾನವಾಗಿ ಪ್ರವೇಶಿಸುವ ರೋಗಕಾರಕಗಳಾಗಿವೆ ಮತ್ತು ತೀವ್ರವಾದ ವೈರಲ್ ಹೆಪಟೈಟಿಸ್ನ ವಿರಳ ಸೋಂಕುಗಳು ಮತ್ತು ಸಾಂಕ್ರಾಮಿಕ ರೋಗಗಳಿಗೆ ಕಾರಣವಾಗುತ್ತವೆ. ಹೆಪಟೈಟಿಸ್ ಬಿ ವೈರಸ್ ಮತ್ತು ಹೆಪಟೈಟಿಸ್ ಸಿ ವೈರಸ್ ಪ್ರಧಾನವಾಗಿ ಪ್ಯಾರೆನ್ಟೆರಲ್ ಮಾರ್ಗದ ಮೂಲಕ ಹರಡುತ್ತವೆ ಮತ್ತು ದೀರ್ಘಕಾಲದ ಹೆಪಟೈಟಿಸ್ಗೆ ಕಾರಣವಾಗುತ್ತವೆ, ಇದು ಯಕೃತ್ತಿನ ಸಿರೋಸಿಸ್ ಮತ್ತು ಹೆಪಟೋಸೆಲ್ಯುಲರ್ ಕಾರ್ಸಿನೋಮ ಸೇರಿದಂತೆ ಗಂಭೀರ ತೊಂದರೆಗಳಿಗೆ ಕಾರಣವಾಗಬಹುದು. ಅಸುರಕ್ಷಿತ ಇಂಜೆಕ್ಷನ್ ಅಭ್ಯಾಸಗಳ ಜೊತೆಗೆ ಕಳಪೆ ರಕ್ತ ವರ್ಗಾವಣೆ ವಿಧಾನಗಳು ಸೋಂಕಿನ ತ್ವರಿತ ಹರಡುವಿಕೆಗೆ ಸಂಭಾವ್ಯ ಮೂಲಗಳಾಗಿವೆ.

ನಿಮ್ಮಿಂದ 3 ಮಿಲಿ ರಕ್ತದ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ನಾವು ಬಯಸುತ್ತೇವೆ. ಈ ಸಂಶೋಧನಾ ಯೋಜನೆ ಮತ್ತು ಇದು ರೋಗಿಗೆ ನೇರವಾಗಿ ಪ್ರಯೋಜನವನ್ನು ನೀಡಬಹುದು ಅಥವಾ ಇಲ್ಲದಿರಬಹುದು, ಕೆಲವು ಸಂಶೋಧನಾ ಪ್ರಶ್ನೆಗಳನ್ನು ಖಚಿತಪಡಿಸಿಕೊಳ್ಳಿ ಅದು ಉತ್ತಮ ರೋಗಿಗಳ ಆರೈಕೆ ಮತ್ತು ಸೂಕ್ತ ಚಿಕಿತ್ಸಾ ತಂತ್ರಕ್ಕಾಗಿ ರೋಗಗಳನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ. ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯು ಕಟ್ಟುನಿಟ್ಟಾಗಿ ಗೌಪ್ಯವಾಗಿರುತ್ತದೆ ಮತ್ತು ಕಾನೂನಿನ ಅಗತ್ಯವಿದ್ದರೆ ಅದನ್ನು ಹೊರತುಪಡಿಸಿ ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ. ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿ ಮತ್ತು ಮಾದರಿಗಳನ್ನು ಸಂಶೋಧನೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಮಾಹಿತಿಯು ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ಬಲವಂತವಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಬಯಸದಿದ್ದರೆ ನಿಮಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಕೊಂಡರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡಬೇಕಾಗುತ್ತದೆ. ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನೀವು ಹಿಂತೆಗೆದುಕೊಳ್ಳುವ ಸ್ವಾತಂತ್ರ್ಯದಲ್ಲಿದ್ದೀರಿ, ಅದು ಸಂಬಂಧಪಟ್ಟ ವೈದ್ಯರಿಂದ ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ರೋಗಿಗಳ ನಿಯತಾಂಕಗಳ ಎಲ್ಲಾ ವಿಶ್ಲೇಷಣೆಯನ್ನು ರೋಗಿಯ ಮೇಲೆ ಗುರುತು ಹೊರೆ ಮಾಡದೆ ಉಚಿತವಾಗಿ ಸಾಗಿಸಲಾಗುತ್ತದೆ. ಈ

ಡಾಕ್ಯುಮೆಂಟ್ ಅನ್ನು ಮೈಕ್ರೋಬಯಾಲಜಿ ವಿಭಾಗದ ಸುರಕ್ಷಿತ ಲಾಕರ್‌ನಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಮಾಹಿತಿಗಾಗಿ ನಿಮಗೆ ನೀಡಲಾದ ಪ್ರತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ.

ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ತನಿಖಾಧಿಕಾರಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ: ಹೆಸರು: ದೇವಿಂದರ್‌ಕೌರ್.

ಸಂಪರ್ಕ ಸಂಖ್ಯೆ: 9056432212.

RECOMMENDATIONS:

Genotypes and subtypes of HCV are important diagnostic target to start the treatment. The viral genotype and IL28B polymorphisms are important factors to personalize antiviral therapy of patients with CHC. This information will aid clinicians to effectively design response-based treatment regimen.