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# PREVALENCE OF HEPATITIS C VIRUS INFECTION AMONG THE ADULT POPULATION OF DISTRICT MARDAN, KHYBER PAKHTUNKHWA, PAKISTAN

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**(SESSION: 2017-2021)**

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# APPROVAL CERTIFICATE

This thesis, submitted by Mr. HAMZA HASSAN, is accepted in its present form by the department of Zoology at Abdul Wali Khan University Mardan, satisfying the thesis requirements for the award of the degree of Bachelor of Studies in Zoology.

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# Dedicated to my beloved parents

# And teachers

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## LIST OF ABBREVIATIONS

HCV hepatitis C virus

NS non structural

ELISA enzyme-linked immuno sorbent assay

IgG immunoglobulin G

IgM immunoglobulin M

ML milliliter

WHO world health organization

HCC hepatocellular carcinoma

ALT alanine amino transferase

HIV human immune deficiency virus

PCR polymerase chain reaction

SVR sustained vital response

MMC mardan medical complex

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It is my first experience in life to acknowledge those people to which I only think and thank in my heart and mind but now is the Opportunity to realize and discuss the role of that people in my present status. In the life of every person there are some forces which direct him on the right way and repel from the wrong one. That may be his family, friends, teachers or physical symbols. When I think about my base of personality so few people came in my mind who are the builders of my base.

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## ABSTRACT

Hepatitis C virus is a Flaviviridae family RNA virus that causes Hepatitis C. The Hepatitis C virus is a major health problem worldwide and remains a vital cause of chronic hepatitis. This study was aimed at detecting the prevalence of HCV infection in the adult population of district Mardan, Khyber Pakhtunkhwa (KP), Pakistan. The blood samples from 300 individuals were evaluated for antibodies using a 3rd generation enzyme-linked immunosorbent assay (ELISA). Out of 300 serum samples, 34.3% were found positive for HCV infection. In Sex-wise study, male showed 33.3% prevalence while female possessed 35.7% of the prevalence of Hepatitis C virus. The overall rate of the hepatitis C virus was high in rural areas 19.6% as matched to the urban areas 14.6%. The overall prevalence of hepatitis C infection in literate patients was lower 14.3% as paralleled to illiterate patients 20%. The overall percentage of hepatitis C infection in married patients was high 23% while in unmarried, patients' prevalence was lower 11.33%. The prevalence of hepatitis C virus was high in age group 41-60 which was 15% while low in age group above 60 which was 7%. Necessary measures should be agreed out in this region towards the control of the infection. The goal of this study was to determine the prevalence of the hepatitis C virus and to educate the public about its harmful effects.

**Keywords**: HCV, ELISA, prevalence, infection, Liver disease, serum, Mardan

# CHAPTER 01

## INTRODUCTION

Hepatitis C is a viral liver disease that affects humans and chimpanzees. It is caused mostly by the Hepatitis C virus. Early on, the infection is often asymptomatic, but once entrenched, it can cause serious liver problems including fibrosis and finally cirrhosis. (Anwar *et al*., 2013). Hepatitis C is one of Pakistan's most frequent chronic infections and a huge healthcare burden (Jafri and Butt, 2008).

Hepatitis C virus was discovered in 1989, allowing research to untangle crucial components of this significant human pathogen's complex life cycle (Bukh, 2016).

About 2% of the world's population is currently infected by the Hepatitis C virus. In Egypt the condition is very serious. There, Hepatitis C virus constitutes an epidemic having highest frequency in the world. There is no other country in the world where Hepatitis C Virus epidemic is affecting the entire population. The prevalence of Hepatitis C virus infection is 1% to 2% in the world. However, it is 14.7% of population in Egypt (Amer *et al*., 2015).

Trends in HCV incidence in the United States were simulated using age-specific occurrence from acute disease reports and age-specific distribution from a cross-sectional nationwide survey. From

late 1960s to the early 1980s, this model revealed a great surge in the frequency of newly developed Hepatitis C (HCV) infections. Before 1965, the estimated annual occurrence was lower (18 per 100, 000), then increased progressively through 1980, and remained high (130 per 100, 000) through 1989, equivalent to an average of 240, 000 cases each year in the 1980s. The number of reported hepatitis C cases has decreased by more than 80% since 1989, which is similar with the result that the national prevalence of infection barely changed between 1988 and 2002. (Alter, 2007).

The world's seventh most prevalent cancer is the primary liver cancer which is the second leading cause of cancer-related death. Asia and Africa have the highest prevalence worldwide. Mongolia has the highest prevalence with 93.7 per 100,000, however, due to a high rate (18.3 per 100,000) and the world's major population, China has the most occurrences (1.4 billion persons).

Hepatocellular carcinoma is the most common kind of liver cancer worldwide, responsible for over 75% of all cases. Hepatocellular carcinoma rates have been declining in some more prevalent areas while rising in several low prevalent areas. Hepatocellular carcinoma rates decreased in several Asian nations and Italy between 1978 and 2012, but grew in India, America, Oceania, and most European countries. However, in recent years, the rise in some nations, like in the United States, has slowed as incidences in various categories have increased or decreased (McGlynn *et al*., 2021).

The occurrence of infection is two to four times hinger in men as compared to women. In United States, the age-adjusted occurrence rate in 2016 was 2.9 per 100,000 in women, whereas, in men it was 10.4 per 100,000. This difference is highest in Europe, where the occurrence rate is four times higher in men than in women (McGlynn *et al*., 2021).

The World Health Organization (WHO) in 2015 predicted 71 million individuals globally and about 10 million from Southeast Asia were suffering from chronic Hepatitis C infection. (Nouhin *et al*., 2019)

The percentage occurrence of Hepatitis C Virus in an area can be estimated by the risk issues which are linked with the transmission of infectious disease. Like the risk factors which contain injection of drugs, blood transfusion, transplantation of organs, hemodialysis, injury, sexual mediation, and vertical spreading.

The Hepatitis C virus infected 1.8 percent (3.9 million) of the US population between 1988 and 1994, according to the Third National Health and Nutrition Examination Survey. Seventy-four percent of the population had noticeable serum Hepatitis C virus RNA, resulting that 2.7 million

Americans have the virus in their blood and are infected with the Hepatitis C virus chronically. Based on previous Hepatitis C virus infection, it has been predicted that before 2015, the number of people infected for more than 20 years will rise dramatically (Yen *et al*., 2003).

With 2 to 3 percent frequency of the Hepatitis C infection in the world, it has been calculated that 130-170 million individuals are infected with hepatitis C virus. The Middle East, Africa are more effected countries with the frequency ranging from 2-15 percent in Cameroon, Iraq, Saudi Arabia, Syria and Egypt. Northern and Western Europe, Australia, Japan and North America has lower frequency of the disease with no nation having occurrence greater than 2 %. India, China, Indonesia and Pakistan contain average half of the world's Hepatitis C virus infected cases. The epidemiological shape of the Hepatitis C virus disease in the Europe is diverse: Northern and Western Europe recorded very low percentage occurrence which is less than 1 percent while Southern and Eastern recorded high percentage occurrence of 2.5 percent (Ansaldi *et al*., 2014).

Hepatitis C frequency predicts from 0.44 to 1.6 percent in kids while 2.4 to 6.5 percent in mature population in majority of disease occurrences (Jafri and Butt, 2007).

## 1.1 Cause

Hepatitis C virus (HCV) is a Flaviviridae noncytopathic hepatotropic infection that infects acute and chronic cirrhosis, as well as hepatocellular cancer. Its major target organ is the liver, and its main targeted cell is the hepatocyte. Hepatitis C virus is Flaviviridae noncytopathic hepatotropic virus that leads to acute and chronic hepatitis as well as hepatocellular cancer. Its major target organ is the liver and its main targeted cell is the hepatocyte (Chisari, 2005).

Hepatitis C virus varies from many other RNA viruses in that it can cause chronic infection and continuous illness in a significant proportion of persons who are infected (Simmonds, 1995)

Hepatitis C virus is a single stranded ribonucleic acid virus that belongs to the family Flaviviridae (Kim and Chang, 2013).

Hepatitis C virus was the first to be identified using molecular cloning rather than biological or biophysical techniques. All the nucleic acids from the plasma of a chimpanzee afflicted with non-A and non-B hepatitis was isolated, copied into complementary DNA and cloned using contaminated factor XIII concentration. Throughout the viral genome, different Hepatitis C virus samples from all around the world display considerable nucleotide sequence variability. HCV has been divided into many strains based on the discovery of these genetic changes. Some of the

variations in disease diagnosis and treatment response found in Hepatitis C virus diseased people are considered to be due to Hepatitis C virus genetic variability (Zein, 2000).

The genome of the Hepatitis C virus is a single-stranded RNA molecule with positive polarity that is 9.6 kilobases long and uncapped. It has 3' and 5' untranslated regions respectively, which include control elements necessary for the translation and duplication. Hepatitis C virus like other persevering viruses does not kill cells it infects but instead inducing an immune-mediated inflammatory reaction that either removes the infection quickly or progressively damages the liver, resulting in Hepatocellular carcinoma. Because Hepatitis C virus spreads quickly in the liver after injection and the inborn immune reaction may have a role in the result of infection. (Chisari, 2005).

One method for examining the fundamental links amongst identified Hepatitis C virus alterations is to use phylogenetic tree analysis of nucleotide sequences from the complete genomes or sub-genomic domains. The diversity of Hepatitis C virus was organized into six equally divergent major groups of sequences and several of which had more closely related groups within them, according to a phylogenetic tree built from the sequencing data. (Simmonds, 1995).

Six main and numerous subtypes of the hepatitis C virus have been identified as a result of variances and mutations in Hepatitis C virus RNA. These genetic forms are the key determinants of antiviral medication duration, interferon choice and the likelihood of a prolonged virological reaction. In North and South America as well as Europe, the most common genotype is 1. In China and Japan, genotype 1 is the most frequent form, whereas genotype 4 is the most common in the Middle East. Genotype 3 is the most common in Pakistan, representing for 67 percent to 87 percent of cases. It's worth noting that genotype 3 is also prevalent in Australia and New Zealand in addition to India, Bangladesh and Pakistan. (Jafri and Butt, 2008)

All of the reported epidemiologically significant Hepatitis C virus variations are still present in genotypes 1 to 6. Subclassifications 1a, 1b, 2a, 2b, 2c, 3a, 4a, 4d, 5a, and 6a have well-defined global or population-specific characteristics. At the nucleotide and extracted amino acid level, the genomes of Hepatitis C virus isolates belonging to various main genotypes differ by around 30 percent while the subtypes are generally different by greater than 15 percent. As a result, the Hepatitis C virus has high amount of the genetic variability across its genome which has the major implications for diagnosis and therapy as well as the development of a vaccine. Hepatitis C virus genotypes appear to have a minor influence on the long-term prognosis of the Hepatitis C virus

infection. As a result, all the genotypes are linked to serious liver disorders and genotype 3 individuals may have a higher risk of developing hepatic steatosis. According to a recent worldwide survey, genotypes 1 and 3 are the most common accounting for 46 percent and 30 percent of all the cases, while genetic types 2, 4, 5, and 6 contributed about 9 percent, 8 percent, 1 percent, and 6 percent of all the illnesses respectively. Genotypes 1, 2, and 3 account for around 90 percent of all the illnesses in Europe but genotype 1 is common in most of the countries while genotype 3 has a substantial frequency in several nations and genotype 2 is common in Italy. Only 50 percent to 73 percent of cirrhotic patients with Hepatitis C virus subtype 3b disease had prolonged virological reaction at 12 weeks after therapy with the Direct Acting Antiviral regimen, according to recent findings from the Asian and Chinese populations (Bukh, 2016).

## 1.2 Pathogenesis

Hepatitis C virus is not cytopathic but liver damage from long-term infection is caused by the host's immunological reaction in an attempt to combat the virus. Acute self-sufficiency powerful and determined multi-specific CD4+ and C8+ T cell responses towards the viral protein epitopes are linked with Hepatitis C virus illness, while the plausible explanations for Hepatitis C virus persistence without such responses include:

1) During Hepatitis C virus replication, the virus undergoes substantial genomic alterations, resulting in many viral species in a single host.

2) mutations that stop proteins from being expressed

3) Subcellular interferon (IFN) signaling is suppressed.

4) fully functioning CD8+ T lymphocyte responses are impaired, and,

5) viruses interfere with the host's defenses. Acute hepatitis C patients are typically non-symptomatic. Although most studies have found significant 77-85 percent rates of development from acute to chronic hepatitis C, the shift from acute to chronic cirrhosis is typically symptom-free and happens in around 5-25 percent of Hepatitis C virally infected individuals over a period of 20-40 years. Hepatocellular carcinoma can occur in as many as 1 to 4 percent of individuals with cirrhosis each year. Cirrhosis is primarily caused by increasing liver fibrosis, the stage of which is the most important prognostic marker in the biological history of chronic hepatitis C.

Patients who have high aminotransferase concentrations and necro-inflammatory activity on a liver biopsy are more prone to develop fibrosis (Antonelli *et al*., 2008). To further understand the Hepatitis C virus window period, three sources of data from donor–recipient situations have been explored. The first is a collection of instances of post-transfusion hepatitis from the late 1970s and early 1980s. Studies of plasma donor seroconversion panels are the second data source on the dynamics of primary Hepatitis C virus. When a frequent plasma donor seroconverts the period and amounts of Hepatitis C virus RNA and Hepatitis C virus core Ag before liver enzymes increase and antibody production can be determined by looking at previous serial donations. As a result of this host-virus interaction, despite the formation of antibodies and CTL to proteins produced across the Hepatitis C virus genome, most of the patients are unable to eliminate the virus and show persistent illness which is frequently accompanied with chronic liver disease (Busch, 2001).

The proteins or peptides transcribed by different sub-genomic regions of the Hepatitis C virus genome, as well as their quasi species, affect the aforementioned process and so play a major part in Hepatitis C viral pathogenesis and sickness aetiology. Hepatitis C virus is a blood borne virus that enters the circulatory system and travels to the liver. At least four host-derived components are required for Hepatitis C virus isolates to enter including scavenger receptor class B type I, Claudin-I, Occluding and CD81. CLDN9 and CLDN6 have also been demonstrated to replace CLDN1 as Hepatitis C virus entry factors in non-liver cells in humans. The CD81 molecule on the surface of host cells functions as a viral receptor, binding to the viral particle and allowing it to enter the liver cell. CD81 is found on the surface of virtually all nucleated cells where it forms a complex with a number of other cell-surface receptors including CD19 and CD21 on B cells to convey a costimulatory signal to the cells. E2 which is considered the viral envelop protein, binds to CD8's main extracellular loop. Hepatitis C virus has a multi-site binding pattern and may attach to numerous different molecules including the low-density lipoprotein receptor, the DC-specific intercellular adhesion molecule 3 grabbing non-integrin and its liver homologue. Because E2 is the most variable viral protein, it has been observed that its interactions with CD81 are strain-specific. Hyper Variable Region-1 and Hyper Variable Region-2 are two highly variable regions that are often mutated presumably as a result of virus-neutralizing antibodies and Hepatitis C virus-specific cytolytic T cells. Hepatitis C virus has a high mutation rate due to its Ribonucleic Acid-dependent Ribonucleic Acid polymerase's lack of proofreading capabilities. As a result, Hepatitis C virus can be found in multiple different but closely related viral species within an infected

person. These are known as Hepatitis C virus quasi species. Hepatitis C virus has the ability to successfully elude innate immunity resulting in long-term viral infection. This happens because Hepatitis C virus has evolved to avoid the immunological assault by blocking the RIG-1 pathway. In the majority of Hepatitis C virally infected individuals, this characteristic is the cause of chronicity. Hepatitis C virus' non-structural proteins, Non Structure3 and Non Structure4A create a complex that activates the Non -Structure protease domain, causing IPS-1 to be cleaved (Irshad *et al*., 2013).

# CHAPTER 02

# REVIEW OF LITERATURE

According to a succession of context of academic, levels of blood alanine aminotransferase, a marker of liver inflammation and hepatocellular damage, decline and even normalize throughout the second and third trimesters of pregnancy (Campion *et al*., 2012).

Hepatitis C virus infects approximately 20 million people in Arab nations now, and without quick and comprehensive action, that figure is expected to skyrocket in the next couple of decades (Daw and Dau, 2012).

Hepatitis C virus is just not a cytopathogenic virus, but the immunological reaction of the host in an effort to combat it causes liver damage owing to long-term inflammation. Autoantibodies are linked to indications of liver damage in those who have been infected. During the last several years, the pathogenetic role of HCV in the production, transit, and clearance from the flow of cry precipitable immune complexes (ICs) has already been intensively investigated in MC. Hepatitis C infection, anti-Hepatitis C virus polymorphic immunoglobulin (Ig)G, and monoclonal immunoglobulin (Ig)M all create ICs that have rheumatoid activity, which is essential for the generation of cryoglobulin and kidney deposition (Antonelli *et al*., 2008).

## 2.1 Symptoms

Hepatitis C is a significant public health issue. Despite the fact that new infections are decreasing, there is still a significant asymptomatic community of people living with the chronic hepatitis C. Many of these patients will acquire clinical signs in the following 10 to 20 years. It is projected that 4 million Americans are afflicted with the virus and that in 2000, hepatitis C would kill more

people than Acquired Immuno Deficiency Syndrome (AIDS). Both hepatitis C and Interferon treatment have been linked to a variety of neuropsychiatric disorders. Furthermore, mental patients are disproportionately infected with the hepatitis C. It's critical for psychiatrists to learn about hepatitis C infection and psychiatric treatment of its consequences. Cirrhosis develops in around 20 percent of chronic carriers with liver failure, portal hypertension, and hepatocellular carcinoma developing as a result. Cirrhosis raises the risk of hepatocellular carcinoma which occurs at a rate of between 1 percent to 4 percent each year after it has developed. Hepatitis C has been linked to a number of extrahepatic symptoms including mixed cryoglobulinemia and glomerulonephritis. Sjogren's syndrome, Porphyria cutaneatarda, hypothyroidism, and hyperthyroidism are all illnesses that are connected. Malaise, exhaustion, and depression are frequent symptoms of hepatitis C in both the acute or chronic stages (Dieperink *et al*., 2000).

Fibromyalgia is the complicated disease characterized by the widespread pain in muscles and joints as well as tenderness at particular anatomic sites known as sensitive spots. Abnormal sleep patterns, fatigue, cognitive impairment and the autonomic symptoms such as lightheadedness, irritable bladder and irritable bowel, are common in patients with fibromyalgia syndrome. Fibromyalgia's aetiology is not fully known, although it's considered to include immune system, hypothalamic-pituitary axis, and sympathetic nervous system dysfunction. Human and animal studies are used to support the idea that cytokines are involved in fibromyalgia. Glial cells are in the central nervous system have cytokine receptors, suggesting that cytokines may play a role in pain regulation in the sympathetic nervous system. The sympathetic nervous system may be activated by cytokines like Inter Leukin-6, and Inter Leukin-8 is known to have a role in sympathetic pain. When compared to control participants, patients with fibromyalgia had higher levels of serum Inter Leukin-2 and higher levels of Inter Leukin-2 production by T lymphatic cells (Thompson and Barkhuizen, 2003).

Critical mixed cryoglobulinemia, vasculitis, sicca symptoms, arthralgia, myalgia, arthritis, and fibromyalgia are all common rheumatologic signs in patients with Hepatitis C virus (HCV) infection. Autoantibodies are frequently seen in patients with long-lasting viral hepatitis c (Kozanoglu *et al*., 2003).

Regardless of the fact that these guiding principles for controlling Hepatitis C virus infection have changed, sadness remains a prevalent condition that might make Hepatitis C viral treatment more challenging. Interferon alfa therapy has been connected to the development of severe depressive

disorders in patients infected with the Hepatitis C virus. Suicides have been documented in people who had never been depressed before. As a result, the depression is still considered relative at the contraindication to the interferon alfa therapy. The incidence of the psychiatric problems including depression is believed to be 20 percent to 30 percent among people infected with the Hepatitis C virus. Recognizing the relationship among the interferon alfa treatment, depression, and Hepatitis C virus infection, the Consensus Statement for Hepatitis C Virus Treatment recommended that depression be assessed and addressed before beginning interferon therapy for Hepatitis C virus infection. As a consequence, developing a reliable depression screening method is crucial. The CES-D and Beck Depression Inventory (BDI) are often used as screening measures for depressive symptoms in community-based and clinic settings. The CES-D was not intended to identify severe depression, despite its ease of use and applicability to community-based populations. On the other hand, the BDI is regarded to be more sensitive to clinical depression, albeit it may overestimate melancholy if physical symptoms linked with Hepatitis C virus infections are prevalent. (Golub *et al*., 2004).

The hepatitis C virus causes both acute and long - term hepatitis. Hepatitis C's clinical course is varied as is the case with many diseases, there is no single normal course or biological history of the disease but rather a wide range of clinical manifestations and consequences. The average time it takes for symptoms to appear is 7 weeks of range, 3 to 20 weeks. However, viral indicators of infection exist long before symptoms arise. Acute, self-limited hepatitis is unfortunately not the most prevalent complication of the acute Hepatitis C virus infection. The majority of patients' acute hepatitis symptoms go away, although ALT levels typically stay high and Hepatitis C virus Ribonucleic Acid remains. Cellular immunity to the Hepatitis C virus antigens may potentially play a role in explaining why 15 percent of patients clear Hepatitis C viral infection while the majority do not (Hoofnagle, 1997).

## 2.2 Transmission

People who inject drugs are the primary component carriers of the hepatitis C virus. Hepatitis C virus transmission is primarily attributable to injectable drug usage in developed nations. There is a strong link between injectable drug usage, the Hepatitis C virus infection, and the incarceration. Because acute Hepatitis C virus infection is often asymptomatic, the exact date and source of

transfer are often unclear. In order to reconstruct possible transmission chains from prevalent cases, viral genotyping and phylogenetic relationships were utilized (Bretaña *et al*., 2015).

Although the Hepatitis C virus is believed to be spread primarily via blood contact, this route does not fully explain how the viruses are transmitted. There has been less confidence about the significance of alternative transmission channels and variables that affect transmission efficiency. The fast speed of development in diagnostic techniques has made assessing Hepatitis C virus transmission difficult. Since the introduction of antibody testing in 1990, the serologic diagnosis of Hepatitis C virus has progressed significantly. In comparison to first-generation tests, second-generation immunosorbent assays (enzyme immunoassay or the enzyme-linked immunosorbent assay) for Hepatitis C virus antibodies have enhanced sensitivity and specificity, as well as shortened the time to detect seroconversion. The existence of tattoos have been linked to an increased risk of Hepatitis C virus illness on its own. Skin piercing and folk medicine are two more possible sources of unnoticed blood contact. One incidence of transmission by mucous membrane contact, via blood splashing to the eye, has also been reported (MacDonald *et al*., 1996).

Vertical transmission is currently the most prevalent route of infection in children, thanks to the introduction of regular blood supply screening. This has practically eliminated transmission by transfusion. In a woman with detectable Hepatitis C virus RNA, the probability of Hepatitis C virus transmission from mother to kid is estimated to be 4.3 percent. Infection with both Hepatitis C virus and Human Immunodeficiency virus raises the chance of vertical transmission by two to three times. The role of manner of delivery on the danger of vertical transmission has been reported in a variety of ways. Cesarean section delivery is not usually advised since it offers no additional advantage in terms of lowering the risk of perinatal transmission. Although the exact timing and mechanism by which the virus is transferred from mother to baby is uncertain, current evidence suggests that transmission is more likely in utero than during the postpartum period (Khaderi *et al*., 2014).

Hepatitis C virus is typically transmitted through parenteral methods. A high number of Hepatitis C virus infections occurred throughout the twentieth century as a consequence of an outbreak of recreational injectable drugs and incorrect injections. The spread of the Hepatitis C virus is an ongoing process that has undergone significant changes over the last century. Certain risk factors, such as the use of illicit intravenous substances and risky behaviors among gay males, make viral transmission are easier and create ideal circumstances for Hepatitis C virus genetic development.

Greater transmission rates occur among acute cases in high-risk populations, resulting in the dissemination of more infectious variations. Hepatitis C virus transmission pathways, on the other hand, might alter over time, affecting Hepatitis CV virus transmissibility. For a variety of reasons, Hepatitis C virus transmission networks are difficult to spot. Recognizing Hepatitis C virus transmission is important for adopting measures to stop the virus from spreading. Fast-evolving viruses, such as Hepatitis C virus, are challenging to track because strains from epidemiologically connected cases are often genetically similar but not identical. Other variables that impact Hepatitis C viral development include co-infection with other viruses and childbearing. This might be linked to the mother's immunological response being altered in some way (Preciado *et al*., 2014).

## 2.3 Risk Factors

### 2.3.1 Alcohol

Liver cancer is one of the most frequent human illnesses and a primary cause of cancer-related death worldwide. Hepatocellular carcinoma which arises from hepatocytes, is by far the most prevalent kind of liver cancer, with rates and etiologies that differ significantly throughout the country (Lambert *et al*., 2011).

Although alcohol use and hepatitis C virus infections are two of the most prevalent causes of liver cirrhosis in Western countries, the combined influence of these two factors on the risk of liver cirrhosis has yet to be well examined. Alcohol use and HCV infection are both risk factors for symptomatic liver cirrhosis with either one being enough to cause the condition. In Italy and other Western nations, alcohol use is the leading cause of liver cirrhosis. The histology of the liver in alcoholics is normal in 5 percent to 25 percent of biopsies and 18 percent of autopsy results while Cirrhosis, on the other hand has a modest annual incidence rate of approximately 2 percent. As a result, only 10% to 25% of alcoholics will develop cirrhosis over their lifetime (Corrao and Arico`, 1998).

Cirrhosis is associated with high mortality rates, and is the main indication for liver transplantation. It is a clinical condition that encompasses people with various causes of chronic liver disease and varying degrees of severity. When cirrhotic individuals with Alcoholic Liver Disease are compared to those with Hepatitis C virus infection, they have a worse survival rate. Other studies have found

that the aetiology of liver illness has little bearing on prognosis. Furthermore, persistent Hepatitis C virus infection may increase the risk of hepatocellular carcinoma more than alcoholic liver disease or nonalcoholic fatty liver disease (Marot *et al*., 2017).

20 percent to 30 percent people infected with hepatitis C virus will develop cirrhosis over the course of two to three decades. It is unclear which viral and host variables have a role in the clinical and histologic development of Hepatitis C virus infection. It has been hypothesized that drinkers who are infected with Hepatitis C virus have higher liver damage. It has been estimated that 5 percent to 25 percent of chronic drinkers with liver damage have a positive Hepatitis C virus antibody result. A series of researches have also found that people with alcoholic liver disease who were infected with the Hepatitis C virus had lower biochemical function and were more likely to develop cirrhosis. (wiley *et al*., 1998).

### 2.3.2 Drug users

Hepatitis C is much more common among people who inject drugs. Among the estimated 9,000 Injecting Drug Users in the area, Amphetamine injection is somewhat more prevalent than heroin injection. Infection with the hepatitis C virus seems to be more frequent in women. Men and women had different Hepatitis C virus seroprevalence rates when the length of injectable drug usage was taken into account. This difference, however, was not substantial (Lidman *et al*., 2009).

### 2.3.3 Coinfection with HIV and HBV

The three most prevalent chronic viral illnesses reported worldwide are the Human Immunodeficiency Virus, the Hepatitis B, and C viruses. Because these viruses spread in similar ways, such as by blood and blood products, sharing needles to inject drugs, and sex acts, co-infection with all these viruses is a typical occurrence. The relevance of the Hepatitis B virus and Hepatitis C virus co-infections in Human Immunodeficiency virus positive persons is critical because of the underlying implications, such as hepatological issues associated with these viruses, which have been found to reduce Human Immunodeficiency Virus infected patients' life expectancy. In the subcontinent, where the average carrier prevalence in the general population is estimated to be 4%, hepatitis B is a serious public health problem (Saravanan *et al*., 2007).

### 2.3.4 Aflatoxin

In terms of liver cancer incidence, the male to female ratio is around 2.4, with the difference being greater in high-incidence areas than in low-incidence ones. According to ecological research, the occurrence of hepatocellular carcinoma is linked to food contamination with aflatoxins, a category of mycotoxins generated by the fungus Aspergillus flavus and Aspergillus parasitic us, which has been shown to induce liver cancer in a variety of experimental animals as well as the Hepatitis B virus and Hepatitis C virus infection. Contamination is most common in Africa, China and Southeast Asia, and is caused by poor storage of cereals, peanuts, and other vegetables. The inadequacy of established techniques of exposure assessment has hampered the research of aflatoxins' carcinogenic function in humans. Aflatoxin B1 exposure is generally a concern in areas where Hepatitis B virus infection is common. Eliminating Aflatoxin B1 exposure may be beneficial to Hepatitis B virus infected people. As a result, a carcinogenic function for aflatoxins, particularly aflatoxin B1, has been shown, which is independent of it and interacts with that exerted by Hepatitis B virus infection.

### 2.3.5 Dietary factors

Several studies have suggested that coffee may have a beneficial influence on liver function and illnesses, including liver cancer. There was additional evidence of an inverse relationship between long-term coffee intake and type 2 diabetes.

### 2.3.6 Obesity and diabetes

Obesity is now well acknowledged as a substantial risk factor for a variety of malignancies. Diabetes, which is linked to obesity, has been suggested as a health risk both for hepatocellular carcinoma and chronic liver disease. Patients with various kinds of liver disease are known to be susceptible to poor glucose tolerance, and it has been hypothesized that the link between hepatocellular carcinoma and diabetes is due to Hepatitis C virus infection. Fatty liver disease, also known as non-alcoholic fatty liver disease, is a condition that may develop to hepatocellular cancer.

### 2.3.7 Oral contraceptives

The use of combination of estrogen and progestogen oral contraceptives increases the incidence of liver adenomas and is linked to the development of hepatocellular carcinoma, while the absolute

risk is expected to be modest and has been demonstrated in populations with low Hepatitis B virus threat. Anabolic steroid usage has been linked to the development of liver cancer in case reports, although the data is not conclusive at this time. Due to the large variance among studies, a recent meta-analysis failed to find a connection between the use of oral contraceptives and the risk of hepatocellular carcinoma (Chuang *et al*., 2009).

## 2.4 Diagnosis

For identifying active Hepatitis C virus illness, nucleic acid testing for the detection of Hepatitis C virus RNA remains the gold standard. However, skilled technical employees, costly equipment and reagents, specialized procedure rooms, and the availability of clean serum or plasma samples are all required in the laboratory setting for performing Nucleic acid testing. Nucleic acid testing is not frequently done in many medical laboratories due to these restrictions. To identify anti-Hepatitis C virus IgG in serum or plasma samples, a number of immunoassays have been established. The first-generation tests used a yeast-expressed recombinant protein with an epitope from the Hepatitis C virus genome's NS4 region. Antigens from the core, NS3 and NS4 regions were included in second and third generation tests, which employed a multiantigen format. The introduction of an extra antigen from the NS5 region distinguishes the second and third generation tests. These assays cut the window time in first generation assays by a mean of 5 weeks, allowing anti-Hepatitis C virus to be identified as early as 10 weeks after infection. Recombinant Immunoblot Assay detects anti-Hepatitis C virus by immobilizing Hepatitis C virus recombinant antigens and synthetic peptides from core, NS3 and NS5 proteins as separate bands onto a membrane. Recombinant Immunoblot Assays have the benefit of being serologic assays that may be run on the same specimen as the screening test and are extremely specific. Rapid tests are usually more costly than traditional immunoassays, and they aren't intended for testing great quantities of material. Hepatitis C virus RNA may be detected in blood or plasma as soon as one week after infection, making it the most accurate marker and golden standard for detecting active Hepatitis C virus illness. Hepatitis C virus genotyping has therapeutic implications since infection with specific genotypes is linked to varied treatment results, which are presently focused on interferon and ribavirin. In trials involving blood donors, identification of Hepatitis C virus core antigen within the first two weeks of acute illness has been observed. For the identification of

biomarkers in a variety of illnesses, including hepatitis C, prototype nanoparticle-based diagnostic tests have been created. Loop-mediated isothermal amplification, a new amplification technique, has the ability to be turned into a moment in time Nucleic acid testing for Hepatitis C virus RNA detection (Kamili *et al*., 2012).

With a competitive enzyme immunoassay, the Hepatitis C virus genotype may be identified by looking for antibodies directed to genotype-specific Hepatitis C virus epitopes. Hepatitis C virus Ribonucleic acid is isolated and reverse transcribed into the double complementary Deoxyribonucleic acid, which is then processed through a cyclic enzymatic procedure, yielding a huge number of identifiable copies. Anti-Hepatitis C virus antibodies should be evaluated by enzyme immunoassay, and Hepatitis C virus RNA should be examined with a sensitive method, such as a Hepatitis C virus RNA assay with a lower detection limit of 50 IU/ml or fewer, in patients suspected of having acute hepatitis C. When both anti-Hepatitis C virus antibodies and Hepatitis C virus RNA (detected using a sensitive method, detecting 50 IU/ml or fewer) are present in individuals with clinical or biological indications of chronic liver disease, chronic hepatitis C is definite (Chevaliez and Pawlotsky, 2006).

Hepatitis C is diagnosed by confirming the existence of hepatitis C virus infection and determining the degree of liver damage. Furthermore, the diagnostic workup should include tests that can assist determine prognosis and treatment response. The detection of viremia is required to confirm the diagnosis of continuing Hepatitis C virus illness. The golden standard for determining the severity of liver disease is liver histology. The only way to identify well compensated cirrhosis is through a liver biopsy. The evidence for a link between blood Hepatitis C virus RNA levels and liver histology is mixed. Many researchers have looked at the link between Hepatitis C virus genotype and liver disease severity. The availability of accurate Polymerase Chain Reaction tests that may serve as the golden standard for the detection of Hepatitis C virus infection clearly influences the decision between the two diagnostic algorithms (Lok and Gunaratnam, 1997).

## 2.5 Treatment

As previously stated, the primary therapeutic aim for chronic Hepatitis C virus illness is to achieve a sustained virologic response. Sustained virologic response can be obtained in around 55 percent of patients with genotype 1 hepatitis C virus infection who get the recommended therapy, and in

80 percent of patients with genotype 2 and 3 hepatitis C virus infection who receive the recommended treatment option.

In HCV-infected individuals, ribavirin monotherapy has little impact. Children who have been infected with HCV for a long time usually have no signs. While biochemistry and histology are similar to adults with Hepatitis C virus, hepatitis C appears to develop more slowly in children than in adults. The majority of side effects are mild to moderate. People who lack the required motivation and therapeutic compliance should be termed untreated. Treatment costs and serious side effects must be balanced against the therapy's limited effectiveness in this population. Hepatic decompensation and kidney failure are also contraindications to therapy with interferon and/or ribavirin. Ribavirin is eliminated by the kidneys. As a result, renal impairment raises ribavirin levels in the blood, worsening adverse effects including hemolysis. Both ribavirin and interferon have a strong impact on the immune system, which is the most likely mechanism of their antiviral action. However, both medications have the potential to exacerbate the illness in Hepatitis C virus patients who also have autoimmune disease. As a result, antiviral treatment should be regarded risky as long as the autoimmune illness is not under control. Hemolysis and anemia are the most prevalent side effects of ribavirin. As a result, individuals receiving combination therapy develop anemia with the lowest hemoglobin four weeks after starting the treatment. The development of autoimmune thyroiditis is the most prevalent autoimmune response to treatment. Interferon-induced flu conditions can be treated with paracetamol or equivalent medicines, while thyroid problems can be addressed with hormone therapy (Weigand *et al*., 2007).

Clinically, it is important to assess the seriousness of the liver illness by evaluating the degree of fibrosis using elastography or the fibrotest, and in certain situations a liver biopsy is required. It is important to distinguish between compensated and decompensated illness in instances of cirrhosis. The existence of extrahepatic Hepatitis C virus symptoms must also be determined. In terms of virology, it is important to identify the genotype and subtype of the hepatitis virus as well as the viral load for which the precise (detection limit of 10 to 15 IU/mL) and easily accessible techniques are advised. Individuals with moderate degrees of fibrosis can avoid treatment, however these patients should be evaluated separately (Grande *et al*., 2016).

Chronic hepatitis C virus illness is linked to intravenous drug use. Hepatitis C virus has been found in 36 percent to 95 percent of intravenous drug users who have been tested for antibodies. Hepatitis

C virus therapy with interferon alfa and ribavirin is still not indicated for individuals who are actively injecting illicit substances or drinking large amounts of alcohol (Backmund *et al*., 2001).

## AIMS AND OBJECTIVES

1. Determine the prevalence of hepatitis C virus infection in the Mardan area.
2. To determine the distribution of hepatitis C virus infection by gender, age, area, education, socioeconomic status, marital status, and employment.
3. Determine the rate of hepatitis C virus infection among smokers and non-smokers.
4. To inform the population for the awareness of transmission and prevention of hepatitis C.

# CHAPTER 03

# MATERIALS AND METHODS

## 3.1 AREA AND LOCATION

This research was carried out in the district Mardan of Pakistan's Khyber Pakhtunkhwa province, which is situated in the south west of the country at 34°12'0N 72°1'60E with an elevation of 283 meters (928 ft). The city is commonly known as city of hostingAfter Peshawar, it is the second-largest city in Khyber Pakhtunkhwa. The title Mardan was granted to a tiny region in honor of Iran's Pir Mardan Shah, a revered spiritual figure.

## 3.2 HISTORY

It is a rapidly expanding metropolis that had a population explosion in the later half of the twentieth century. The region near Mardan was part of the Gandhara burial culture's heartland approximately 1800 BCE. It is situated in an archaeologically rich area. The Sanghao Caves, located outside of Mardan, were found in 1962 and produced artifacts dating back to the Middle Paleolithic era, about 30,000 years ago. Around 1800 BCE, the region around this city became part of the Gandhara grave culture's heartland.

### 3.3 CLIMATE

This city has a hot semi-arid climate that is influenced by the nearby steppe climate. The summer season is very hot. From May to June, there is a significant increase in temperature. Even in July, August, and September, temperature is extremely high. From October onwards, low temperatures have been recorded. December and January are the coldest months throughout the year. The average minimum temperature in January is 0.5 degrees (32.9 degrees Fahrenheit).

### 3.4 DEMOGRAPHICS

This city has a population of 2.25 million people. It has 358,604 residents, making it the second-largest city in Khyber Pakhtunkhwa, according to the 2017 Pakistan Census. This city's population was distributed among 45,429 homes, with an average household size of 7.89 people. It witnessed rapid expansion in the second part of the twentieth century, with the population more than doubling in just 50 years.

### 3.5 OCCUPATIONS

In Mardan, the majority of residents work as farmers. The main occupation in this city is agriculture. People are either directly or indirectly involved in the field of agriculture. The creation of Enterprises in various areas of the district has resulted in an increase industrial work. Some people work in private sectors as well as in government organizations for their living.

### 3.6 EDUCATION

There was no public or private sector university in Mardan till 2009. The first public sector university, it was established in 2009. Since then, education has improved significantly, yet the standards in governmental funded schools are still low in comparison with the rest of country. The education level is 49.95 percent among the people aged 10 and above.

### 3.7 METHAOLOGY

### 3.8 SAMPLE SIZE

Hepatitis C infection is a liver disease characterized by the presence of hepatitis C virus in blood serum of the patient. To determine the seroprevalence of hepatitis C in general population of District Mardan, blood samples were collected from 300 individuals attending Mardan Medical complex (MMC) for evaluation.

### 3.9 STUDY DESIGN

The nature of study was a lab work. All the data has been collected from Mardan Medical Complex in the seven months period started from February 2021 and ends to August 2021. Questionnaire was made which was filled according to the requirements of the project. The study design is

ethically corrected because the questionnaire was filled by the permission of both the patient and medical staff of the hospital. All the 300 serum samples were examined in the MMC laboratory out of which 103 were positive for hepatitis C infection and the remaining were negative.

### 3.10 STUDY PERIOD

The data were collected from February 2021 To August 2021

### 3.11 QUESTIONNAIRE

A questionnaire was used to assess the patients. The questionnaire was created to be applied to patients and was later decided interview. The questionnaire was filled voluntary and on the permission of both the hospital and patient. The questionnaire consists of 12questions in which 7 questions are related to laboratory diagnosis and symptoms, and 5 questions are about personal, economic, and social status of the family. Different answers were allocated to the questions, such as yes, no, zero, or number, and so on. The study period was four months and the questionnaire was filled in that period.

### 3.12 TEST PROTOCOL

#### 3.12.1 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TEST

A serologic test is done for the screening of the subject's serum sample. The test is performed by enzyme-linked immunosorbent assays (ELISA), which detect the presence of hepatitis C antibodies in blood. The result of the test is reported as either positive or negative. Third-generation EIAs have a specificity of approximately 99 percent.

#### 3.12.2 MATERIALS REQUIRED

- Distilled water
- Gloves
- Timer
- Pipette
- Multi-pipette channel

- Kits (containing all the chemicals)
- Cardboard
- Incubator

### 3.12.3 CHEMICALS REQUIRED

- Simple diluent
- Enzyme control
- Stop solution
- Chromogen (TMB).

## 3.12.4 SAMPLE COLLECTION AND PROCESSING

1. A total of 3 ml of blood is collected from the upper superficial vein of the arm of each individual with the help of sterilized syringes. To do that, a laboratory professional will:
2. 1. skin-cleansing
3. To make the veins bulge with blood, an elasticated bandage (tourniquet) is placed over the spot.
4. 3A needle is placed into a vein (typically on the palm of the hand or inside the elbow).
5. 4. Fill a vial or syringe with the blood sample.
6. 5. The needle is removed from the vein, and the flexible bandage is removed.

### 3.12.5 PREPARATION OF CHEMICALS

Washing solution is formed by mixing washing buffer about 1 ML and D/W about 20 ML. Washing is formed by mixing conjugate diluent R6 and R7. Enzyme development solution TMB is formed by adding 1 ML of diluent chromogen (R6) with 10 ML of substrate buffer (R8).

### 3.12.6 PROCEDURE

**Specimen** Blood is drawn into a tube containing an anticoagulant (usually etylene diamine triacetic acid) to prevent it from naturally clotting.

After that, the specimen is transferred to a laboratory for analysis. A technique to detect the presence of antigens in biological samples. Testing is usually done on an automated analyzer, but abnormal results can also be investigated.

### 3.12.7 PRINCIPLE

ELISA stands for enzyme immunoassay in solid phase, and it is used to identify the presence of a certain protein (antigen or antibody) in samples taken. The fundamental idea behind ELISA is to utilize an enzyme to detect antigen (Ag) or antibody binding (Ab). ELISA is based on three main concepts which are:

(i) The existence of Ag or Ab in a sample is identified in an antigen-antibody response.

(ii) the enzymatic chemical process in which the amount of Ag or Ab involved in a chemical reaction is determined by the rate of production of the Ag-Ab complex. The enzyme catalyzes a reaction between the colorless substrate and the colorful result.

(iii) The intensity of the colored product formed by the enzyme and substrate is detected and evaluated in signal detection and quantification.

### 3.12.8 FORMULA TO FIND PREVALENCE

With the aid of the following formula, the prevalence rate was calculated as a frequency;

$$\text{Prevalence (as \%)} = 100 \times \frac{Number of Positive Samples}{Number of Total Samples Measured}$$

# CHAPTER 04

## RESULTS

The frequency of hepatitis C virus infection in the adult population of District Mardan was investigated in this research. A total of 300 serum samples were collected from Mardan Medical Complex (MMC) located in the district. The prevalence was detected in the patients with their name, age, gender, address, area, socioeconomic status, marital status, education, occupation and on the basis of smoking.

**Table 4.1 HCV prevalence in District Mardan as a whole**

| | Total | Positive | Prevalence (Positive) | Negative | Prevalence (Negative) |
|---|---|---|---|---|---|
| HCV | 300 | 103 | 34.3 % | 197 | 65.6 % |

A total of 300 serum samples were recorded for hepatitis C virus infection in which 103 were positive with the percentage of 34.3 % and 197 were negative with the percentage of 65.6 %.

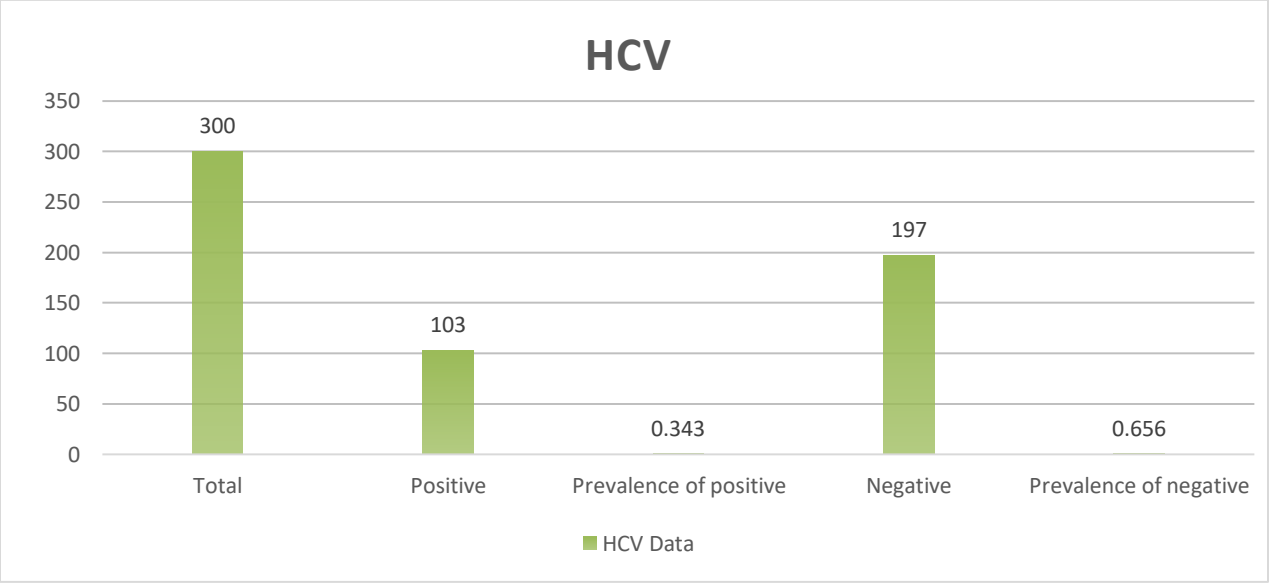


**Fig 4.1 HCV prevalence in District Mardan as a whole**

**Table 4.2 Prevalence of HCV by Gender**

| Gender | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Male** | 177 | 59 | 33.3 % | 118 | 66.6 % |
| **Female** | 123 | 44 | 35.7 % | 79 | 64.2 % |

A combination of 177 blood samples were obtained from men, with 59 of them being positive with a frequency of 33.3 percent, and 123 samples from females, with 79 being positive with a prevalence of 64.2 percent.

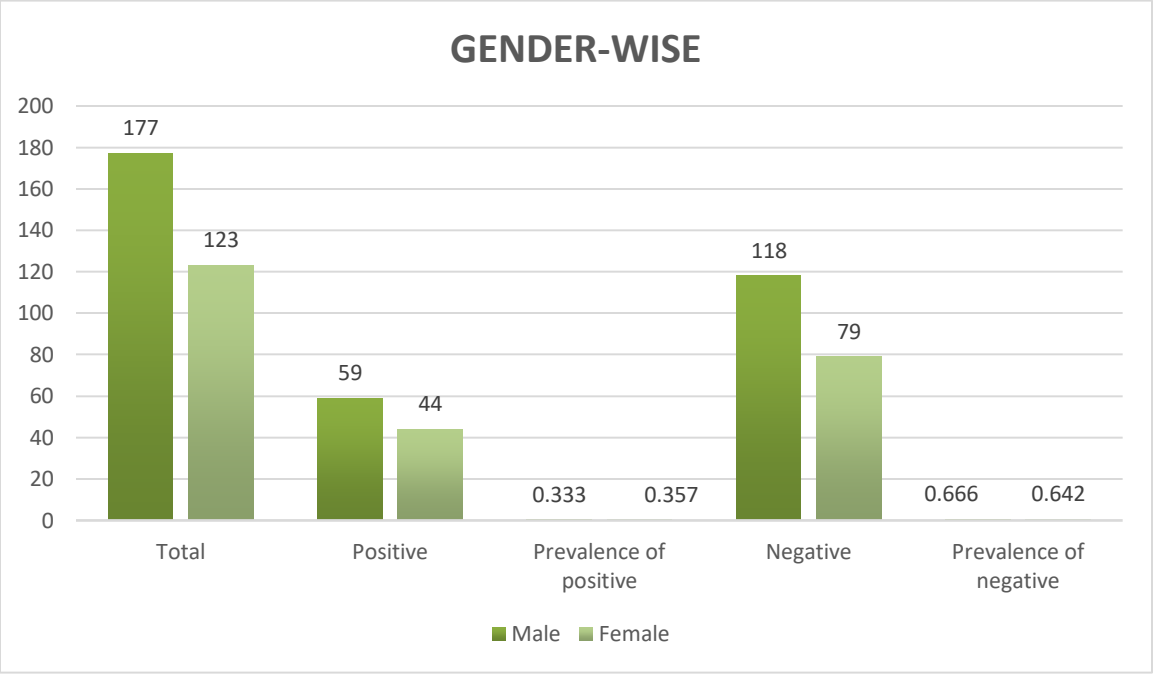


**Figure 4.2 Prevalence of HCV by Gender**

**Table 4.3 HCV prevalence by region**

| Area | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Rural** | 154 | 59 | 19.6 % | 95 | 31.6 % |
| **Urban** | 146 | 44 | 14.6 % | 102 | 34 % |

A total of 154 blood samples were collected in rural area in which 59 were positive with the percentage of 19.6 % while 146 samples were collected from urban area in which 44 were positive having the percentage of 14.6 %.

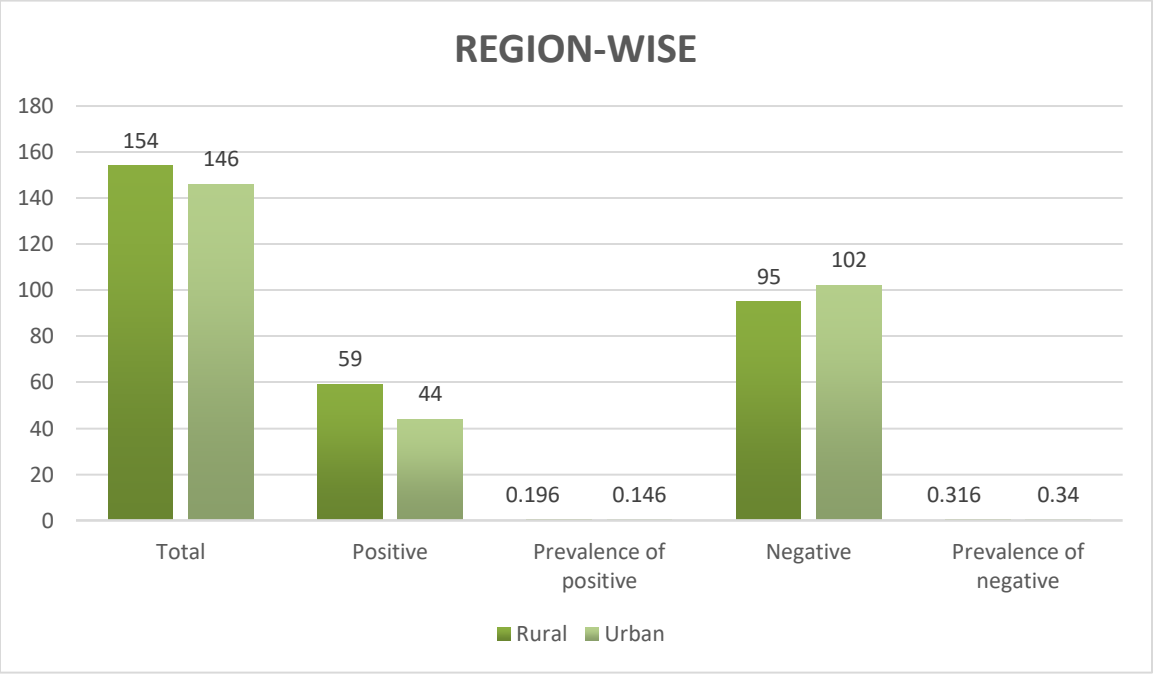


**Figure 4.3 HCV prevalence by region**

**Table 4.4 HCV prevalence by Educational level**

| Education | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Literate** | 154 | 43 | 14.3 % | 111 | 37 % |
| **Illiterate** | 146 | 60 | 20 % | 86 | 28 % |

A total of 154 serum samples were taken from literate persons, of which 43 were positive with such a 14.3% frequency, and 146 serum swabs were analyzed from illiterate people, of which 60 were positive with a 20% prevalence.

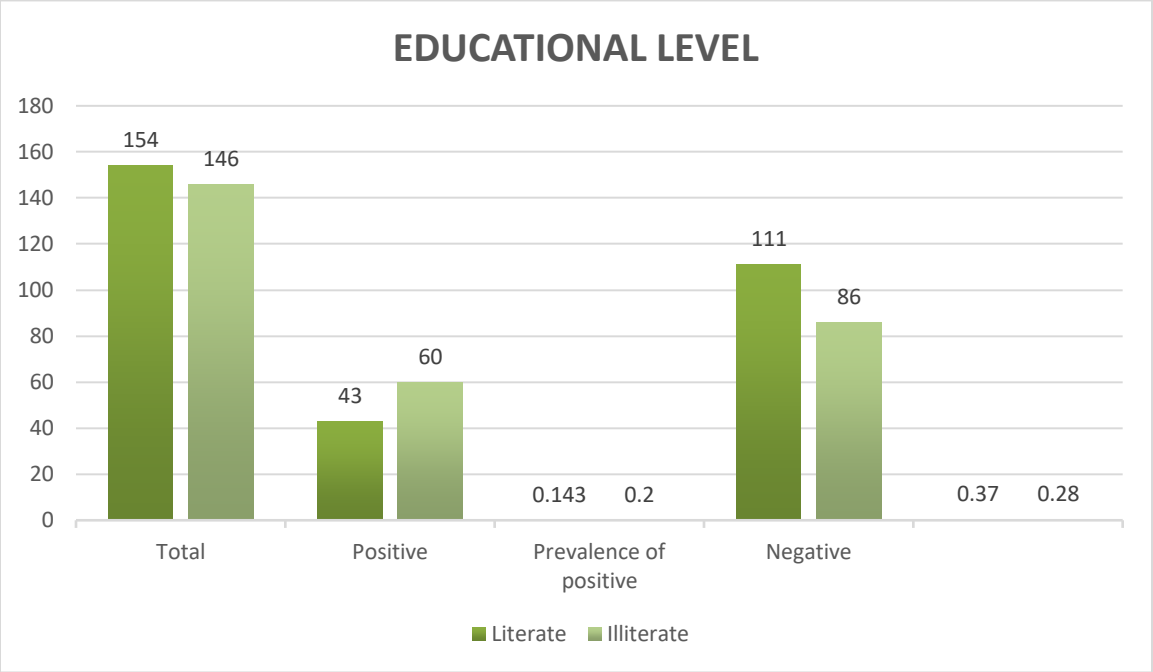


**Figure 4.4 HCV prevalence by Educational level**

**Table 4.5 Marital status-wise prevalence of HCV**

| Marital status | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Married** | 189 | 69 | 23 % | 120 | 40 % |
| **Unmarried** | 111 | 34 | 11.33 % | 77 | 25.66 % |

A combination of 189 serum samples were taken from married persons, with 69 being positive with a percentage of 23 percent, and 111 serum samples from unmarried people, with 34 being positive with a percentage of 25.66 percent.

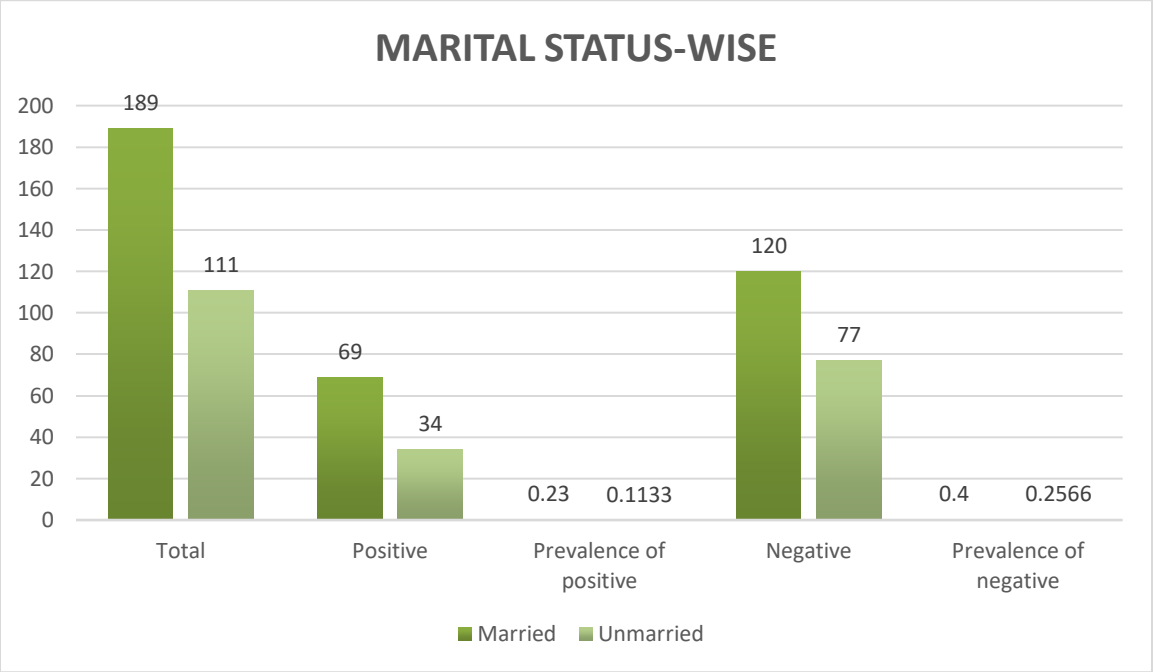


**Figure 4.5 marital status-wise prevalence of HCV**

**Table 4.6 Age-wise prevalence of HCV**

| Age | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **20-40** | 99 | 37 | 12.3 % | 62 | 20.6 % |
| **41-60** | 124 | 45 | 15 % | 79 | 26.3 % |
| **Above 60** | 77 | 21 | 7 % | 56 | 18.6 % |

A maximum of 99 blood samples were taken from people aged 20 to 40, with 37 of them being positive, indicating a frequency of 12.3%. From age group 41-60, 124 total samples were collected in which 45 were positive having the percentage of 15 %. Above age 60 total 77 samples which examined in which 21 were positive having the percentage of 7 %.

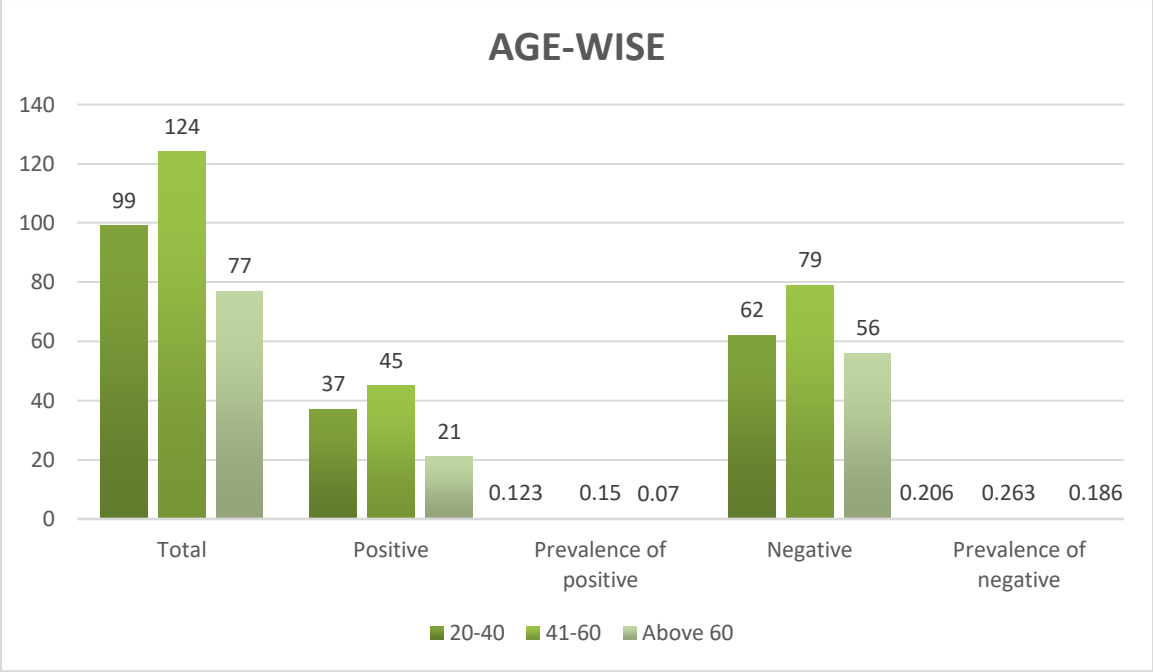


**Figure4.6 Age-wise prevalence of HCV**

**Table 4.7 Socioeconomic status-wise prevalence of HCV**

| Economic Level | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Upper class** | 39 | 09 | 3 % | 30 | 10 % |
| **Middle class** | 119 | 28 | 9.33 % | 91 | 30.33 % |
| **Lower class** | 142 | 66 | 22% | 76 | 25.33 % |

A total of 39 samples were taken from the upper class, with nine positive samples accounting for 3% of the total. Total of 119 samples were collected from the middle class having 28 positive samples with the percentage of 9.33 %. At last, 142 samples were collected from the lower class having 66 positive samples with the percentage of 22 %. Lower class people had the highest prevalence rate.

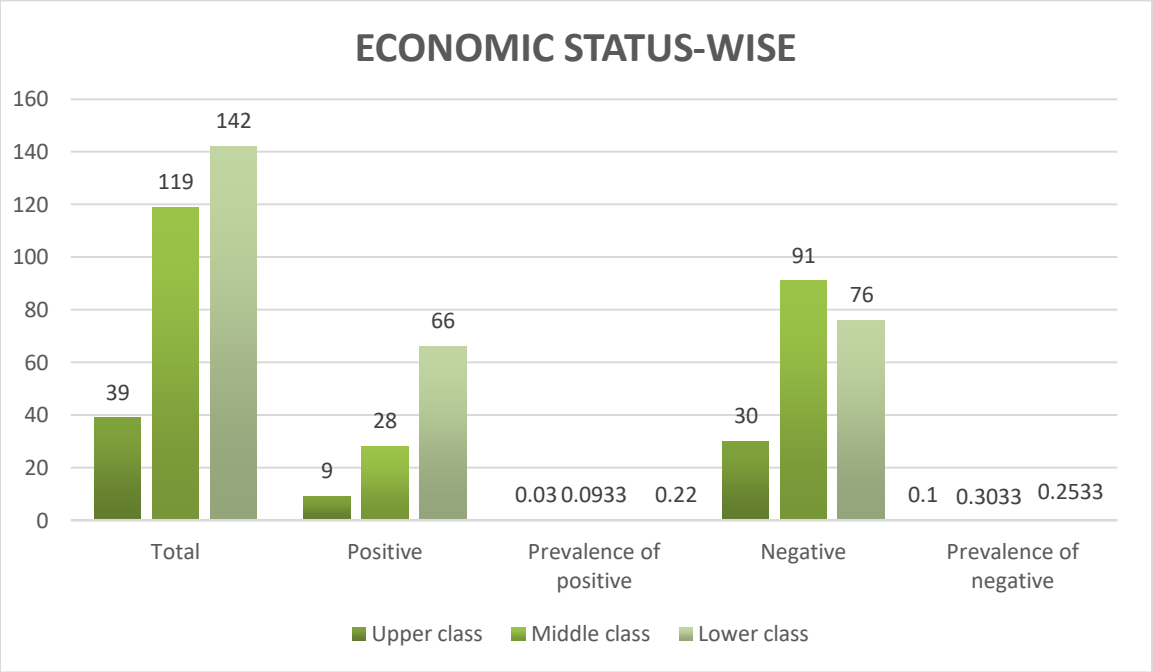


**Figure 4.7 Economic status-wise prevalence of HCV**

**Table 4.8 Prevalence of HCV on the basis of smoking**

| Smoking | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Smoker** | 179 | 67 | 22.33 % | 112 | 37.33 % |
| **Non-smoker** | 121 | 36 | 12 % | 85 | 28.33 % |

A total of 179 samples were collected from current smokers, of which 67 were positive with a prevalence rate of 22.33 percent, while 36 samples were positive with a prevalence rate of 12 percent among non-smokers.

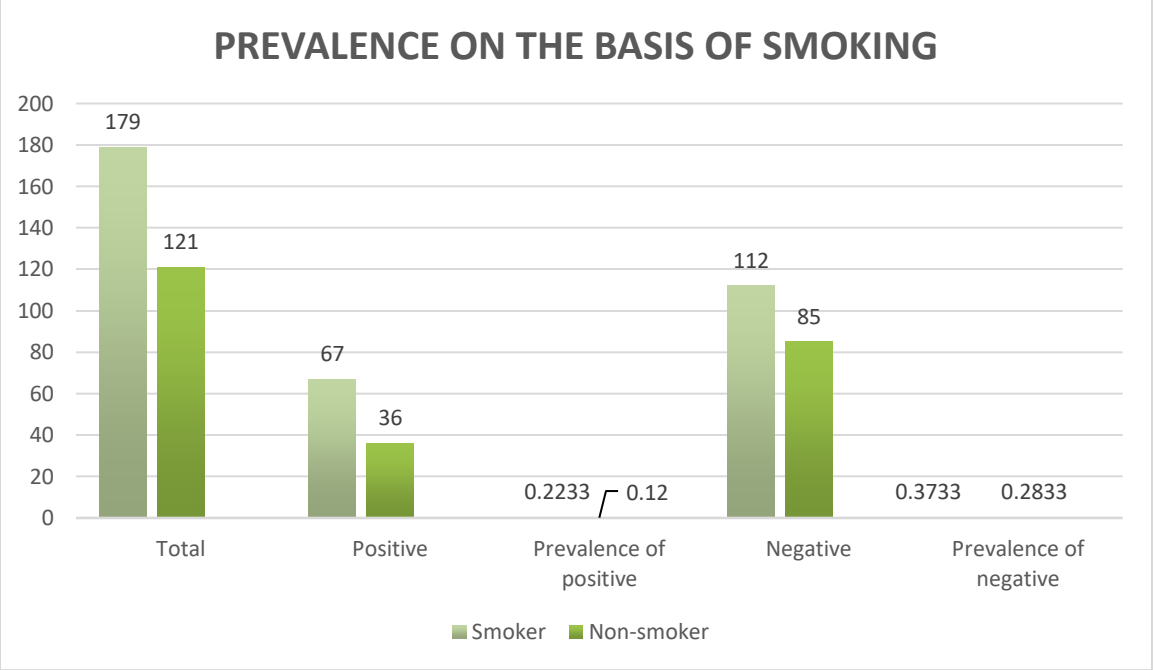


**Figure 4.8 Prevalence of HCV on the basis of smoking**

**Table 4.9 Occupation-wise prevalence of HCV**

| Occupation | Total | Positive | Prevalence of positive | Negative | Prevalence of negative |
|---|---|---|---|---|---|
| **Jobless** | 89 | 17 | 5.66 % | 72 | 24 % |
| **Job** | 211 | 86 | 28.66% | 125 | 41.66 % |

In a total of 300 samples, 89 people are unemployed and 211 are employed. About 17 unemployed people, or 5.66 percent, are infected with the Hepatitis C virus, whereas 72 unemployed people, or 24 percent, have negative samples.

About 86 people who work are infected with the hepatitis C virus and have a 28.66 percent positive rate, whereas 125 people have a 41.66 percent negative rate.

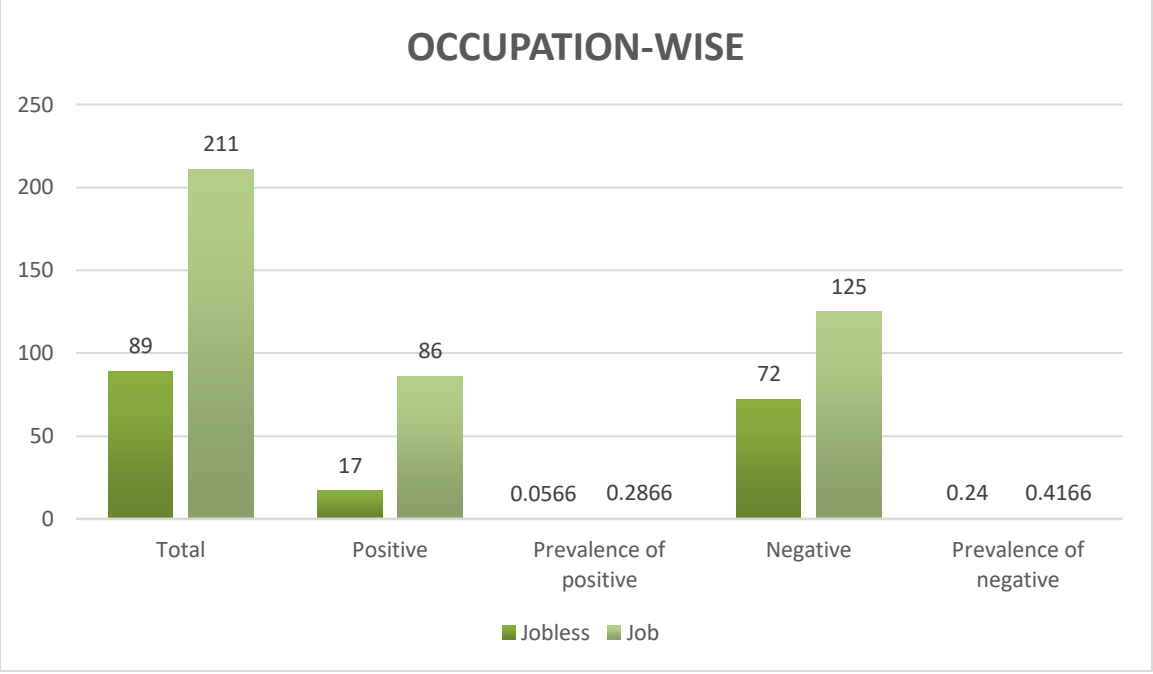


**Figure 4.9 Occupation-wise prevalence of HCV**

# CHAPTER 05

## DISCUSSION

Hepatitis C is an infection of the liver, the Hepatitis C virus infection is typically asymptomatic, and hepatocellular carcinoma, cirrhosis, and end-stage liver damage are the results of the persistent infection (Waqas *et al*., 2015).

This study was performed by collecting 300 serum samples from Mardan Medical Complex (MMC), in which 103 samples having the percentage of 34.3% are HCV positive and 197 samples with the prevalence rate of 65.6% are HCV negative. In Mardan, 14.32 percent of people have Hepatitis C virus infection (Zeeshan *et al*., 2019). The total percentage of Hepatitis C virus was 20.8 percent in 356 serum samples (Khan *et al*., 2013) which is lesser than the recent study. It is because of transmission of infection, improper dental and surgical procedure, lack of education and awareness.

The current study elaborates that about 177 are males and 123 are females, in which 33.3% males are positive for hepatitis C virus infection while 35.7% females are infected. About 59.9% males and 53.6% females got the hepatitis C viral infection (Wasim *et al*., 2014) which is significantly greater than the findings of our research. It is because of infrequent injection of drug use and improvement in the awareness of people since 2014. The females are affected more because they share most of their items with each other.

In area-wise comparison between rural and urban, rural are more infected than urban areas. According to Guerra *et al*., 2011 that 18.3 percent population in rural regions is infected with hepatitis C virus infection and 10.3 percent population is positive in urban areas. In the current study, 19.6% are HCV positive that belong to the rural areas while 14.6% are HCV positive which are from urban areas.

This study concludes that rural population is more infected from hepatitis C virus than the urban population. The current study indicates higher prevalence than the previous study. Razor blades, body piercing, surgical and dental techniques and reuse of unhygienic injections for medical

purposes were the major components which were noticed more in the rural areas as compared to urban region. In rural areas when people often use drugs without consultation with doctors which makes higher risk for the transmission of disease.

Illiterate people are more susceptible to hepatitis C infection than educated ones. According to the latest study, the frequency of hepatitis C infection is greater in the illiterate population than in the educated one. In recent study 43 literate individuals are positive for the hepatitis C viral infection. Out of 154 having the prevalence of 14.3% while in illiterate population, 60 people out 146 are infective with the prevalence of 20%. According to Daw *et al*., 2014 the frequency of Hepatitis C virus was significantly greater in the uneducated group which was 3.1%, whereas it varied from 0.9 to 1.1 percent in the literate group. The major reason of the high prevalence rate is the lack of education and lack of awareness among the illiterate population. Unfortunately, in Pakistan most of the people are unaware of the knowledge of the widespread of the hepatitis C virus infection and other diseases. Thus, the outcome of illiterate population is serious, alarming and potentially life-threatening.

Recent study shows the prevalence rate of Hepatitis C virus infection on the basis of different age groups. The individuals of age group 41-60 have the prevalence of 15% which is higher than any other age group. The age group 41-50 has the higher prevalence rate of 14.28% (Ali *et al*., 2010) which is quite similar having smaller difference. It is because of smoking and Naswar (tobacco) which is quite common in the individuals of the specified age groups.

The current study evaluates the prevalence rate of hepatitis C virus infection in the population on the basis of smoking. In recent study the prevalence of hepatitis C virus infection in individuals who were smokers is 22.33% while 12% prevalence is recorded in people who are non-smokers.

Heavy smoking results in the severity of hepatic lesions when associated with hepatitis C virus (HCV) infection. All 170 patients tested positive for hepatitis C viral RNA in which 43 patients with the prevalence of 25% were smokers (Dev *et al*., 2006) which is higher than the recent study. It maybe because different organizations in the country are trying different ways to aware the people regarding the dangers of smoking and hepatitis C. The government has taken preventive

and strict measures against tobacco use and has also banned smoking in public places and workplaces.

The purpose of this study was to find out how common hepatitis C is in patients based on their name, gender, address, location, age group, socioeconomic status, education, smoking, blood transfusion, occupation, marital status, and addiction, as well as to identify the potential route of septicity among patients.

## CONCLUSION

Hepatitis C is the most widespread infection in our culture. The overall prevalence of hepatitis C virus infection was found to be 34.3% in the district of Mardan. This study concludes that hepatitis C is high in females (35.7%) than in males (33.3%). The females are more effected because they mostly use each other's individual items. Illiteracy is a major factor in the high prevalence rate of this illness in rural areas. Majority of individuals in rural areas have unproductive awareness regarding hepatitis C infection and the way of its transmission and prevention. Academic establishments and the media have a critical influence in changing people's perceptions about the hepatitis C virus.

## RECOMMENDATIONS

Following are some of the recommendations for the Hepatitis C viral disease;

- It is important to rais the public awareness of this disease, particularly among individuals who live in rural regions. Hepatitis C virus infection can thus be reduced by taking such courses.
- Personal items such as razors and toothbrushes should not be distributed among each other to prevent the spread of this illness.
- If at all possible, avoid contact with blood and blood products.
- Drug barbs and other drug preparations (such as grunting drugs grasses) should never be shared to avoid the spread of this disease.
- Disemboweling blood tumbles should be done using a mixture of one part household bleach to nine parts water.
- Individuals who have sex outside of a monogamous relationship should participate in safer sex practices to avoid obtaining hepatitis C virus infection.

# CHAPTER 06

## REFERENCES

Ali, A., Ahmad, H., Ali, I., Khan, S., Zaidi, G., & Idrees, M. (2010). Prevalence of active hepatitis c virus infection in district mansehra Pakistan. *Virology Journal*, *7*(1), 1-5.

Alter, M.J., 2007. Epidemiology of hepatitis C virus infection. World journal of gastroenterology: WJG, 13(17), p.2436.

Amer, F.A., Gohar, M. and Yousef, M., 2015. Epidemiology of hepatitis C virus infection in Egypt. International Journal of Tropical Disease & Health, pp.119-131.

Ansaldi, F., Orsi, A., Sticchi, L., Bruzzone, B., & Icardi, G. (2014). Hepatitis C virus in the new era: perspectives in epidemiology, prevention, diagnostics and predictors of response to therapy. *World journal of gastroenterology: WJG*, *20*(29), 9633.

Antonelli, A., Ferri, C., Galeazzi, M., Giannitti, C., Manno, D., Mieli-Vergani, G., Menegatti, E., Olivieri, I., Puoti, M., Palazzi, C. and Roccatello, D., 2008. HCV infection: pathogenesis, clinical manifestations and therapy. Clinical and experimental rheumatology, 26(2), p.S39.

Anwar, M.I., Rahman, M., Hassan, M.U. and Iqbal, M., 2013. Prevalence of active hepatitis C virus infections among general public of Lahore, Pakistan. Virology journal, 10(1), pp.1-6.

Backmund, M., Meyer, K., Von Zielonka, M. and Eichenlaub, D., 2001. Treatment of hepatitis C infection in injection drug users. Hepatology, 34(1), pp.188-193.

Bretaña, N.A., Boelen, L., Bull, R., Teutsch, S., White, P.A., Lloyd, A.R. and Luciani, F., 2015. Transmission of hepatitis C virus among prisoners, Australia, 2005–2012. Emerging infectious diseases, 21(5), p.765.

Bukh, J., 2016. The history of hepatitis C virus (HCV): Basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. Journal of hepatology, 65(1), pp.S2-S21.

Busch, M.P., 2001. Insights into the epidemiology, natural history and pathogenesis of hepatitis C virus infection from studies of infected donors and blood product recipients. Transfusion clinique et biologique, 8(3), pp.200-206.

Chevaliez, S. and Pawlotsky, J.M., 2006. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. International journal of medical sciences, 3(2), p.35.

Chisari, F.V., 2005. Unscrambling hepatitis C virus-host interactions. NATURE-LONDON-, 7053, p.930.

Chuang, S.C., La Vecchia, C. and Boffetta, P., 2009. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. Cancer letters, 286(1), pp.9-14.

Corrao, G. and Aricò, S., 1998. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. Hepatology, 27(4), pp.914-919.

Daw, M.A. and Dau, A.A., 2012. Hepatitis C virus in Arab world: a state of concern. *The Scientific World Journal*, *2012*.

Daw, M.A. and El-Bouzedi, A., 2014. Prevalence of hepatitis B and hepatitis C infection in Libya: results from a national population based survey. *BMC infectious diseases*, *14*(1), pp.1-9.

Dev, A., Patel, K., Conrad, A., Blatt, L.M. and McHutchison, J.G., 2006. Relationship of smoking and fibrosis in patients with chronic hepatitis C. *Clinical Gastroenterology and Hepatology*, *4*(6), pp.797-801.

Dieperink, E., Willenbring, M. and Ho, S.B., 2000. Neuropsychiatric symptoms associated with hepatitis C and interferon alpha: a review. American Journal of Psychiatry, 157(6), pp.867-876.

Golub, E.T., Latka, M., Hagan, H., Havens, J.R., Hudson, S.M., Kapadia, F., Campbell, J.V., Garfein, R.S., Thomas, D.L. and Strathdee, S.A., 2004. Screening for depressive symptoms among

HCV-infected injection drug users: examination of the utility of the CES-D and the Beck Depression Inventory. Journal of Urban Health, 81(2), pp.278-290.

González-Grande, R., Jiménez-Pérez, M., Arjona, C.G. and Torres, J.M., 2016. New approaches in the treatment of hepatitis C. World journal of gastroenterology, 22(4), p.1421.

Guerra, J., Garenne, M., Mohamed, M.K. and Fontanet, A., 2012. HCV burden of infection in Egypt: results from a nationwide survey. *Journal of viral hepatitis*, *19*(8), pp.560-567.

Hoofnagle, J.H., 1997. Hepatitis C: the clinical spectrum of disease. Hepatology, 26(S3), pp.15S-20S.

Irshad, M., Mankotia, D.S. and Irshad, K., 2013. An insight into the diagnosis and pathogenesis of hepatitis C virus infection. World journal of gastroenterology: WJG, 19(44), p.7896.

Jafri, W. and Subhan, A., 2008. Hepatitis C in Pakistan: Magnitude, Genotype, Disease Characteristics and Therapeutic. Tropical Gastroenterology, 4, pp.194-201.

Kamili, S., Drobeniuc, J., Araujo, A.C. and Hayden, T.M., 2012. Laboratory diagnostics for hepatitis C virus infection. Clinical infectious diseases, 55(suppl_1), pp.S43-S48.

Khaderi, S., Shepherd, R., Goss, J.A. and Leung, D.H., 2014. Hepatitis C in the pediatric population: transmission, natural history, treatment and liver transplantation. World Journal of Gastroenterology: WJG, 20(32), p.11281.

Khan, A., Tareen, A.M., Ikram, A., Rahman, H., Wadood, A., Qasim, M. and Khan, K., 2013. Prevalence of HCV among the young male blood donors of Quetta region of Balochistan, Pakistan. *Virology journal*, *10*(1), pp.1-4.

Khan, M. A., & Rasheed, M. R. (2020). Electronic Media and Interpersonal Discourse: Mediation in Crises. Journal of Social Sciences & Humanities, 28(1)

Kim, C.W. and Chang, K.M., 2013. Hepatitis C virus: virology and life cycle. Clinical and molecular hepatology, 19(1), p.17.

Kozanoglu, E., Canataroglu, A., Abayli, B., Colakoglu, S. and Goncu, K., 2003. Fibromyalgia syndrome in patients with hepatitis C infection. Rheumatology International, 23(5), pp.248-251.

Lambert, M.P., Paliwal, A., Vaissière, T., Chemin, I., Zoulim, F., Tommasino, M., Hainaut, P., Sylla, B., Scoazec, J.Y., Tost, J. and Herceg, Z., 2011. Aberrant DNA methylation distinguishes hepatocellular carcinoma associated with HBV and HCV infection and alcohol intake. Journal of hepatology, 54(4), pp.705-715.

Le Campion, A., Larouche, A., Fauteux-Daniel, S. and Soudeyns, H., 2012. Pathogenesis of hepatitis C during pregnancy and childhood. Viruses, 4(12), pp.3531-3550.

Lidman, C., Norden, L., Kåberg, M., Käll, K., Franck, J., Aleman, S. and Birk, M., 2009. Hepatitis C infection among injection drug users in Stockholm Sweden: prevalence and gender. Scandinavian journal of infectious diseases, 41(9), pp.679-684.

Lok, A.S. and Gunaratnam, N.T., 1997. Diagnosis of hepatitis C. Hepatology, 26(S3), pp.48S-56S.

MacDonald, M., Crofts, N. and Kaldor, J., 1996. Transmission of hepatitis C virus: rates, routes, and cofactors. Epidemiologic reviews, 18(2), pp.137-148.

Marot, A., Henrion, J., Knebel, J.F., Moreno, C. and Deltenre, P., 2017. Alcoholic liver disease confers a worse prognosis than HCV infection and non-alcoholic fatty liver disease among patients with cirrhosis: An observational study. PloS one, 12(10), p.e0186715.

McGlynn, K.A., Petrick, J.L. and El-Serag, H.B., 2021. Epidemiology of hepatocellular carcinoma. Hepatology, 73, pp.4-13.

Nouhin, J., Iwamoto, M., Prak, S., Dousset, J.P., Phon, K., Heng, S., Kerleguer, A., Le Paih, M., Dussart, P., Maman, D. and Rouet, F., 2019. Molecular epidemiology of hepatitis C virus in Cambodia during 2016–2017. Scientific reports, 9(1), pp.1-9.

Preciado, M.V., Valva, P., Escobar-Gutierrez, A., Rahal, P., Ruiz-Tovar, K., Yamasaki, L., Vazquez-Chacon, C., Martinez-Guarneros, A., Carpio-Pedroza, J.C., Fonseca-Coronado, S. and Cruz-Rivera, M., 2014. Hepatitis C virus molecular evolution: transmission, disease progression and antiviral therapy. World journal of gastroenterology: WJG, 20(43), p.15992.

Saravanan, S., Velu, V., Kumarasamy, N., Nandakumar, S., Murugavel, K.G., Balakrishnan, P., Suniti, S. and Thyagarajan, S.P., 2007. Coinfection of hepatitis B and hepatitis C virus in HIV-infected patients in south India. World journal of gastroenterology: WJG, 13(37), p.5015.

Shereen, A., Azam, M., Khan, A., Tunio, S.A., Ullah, S., Khan, M.I., Ullah, H. and Sarwar, T., 2014. Prevalence of active HCV Infection and identification of its common Genotypes in District Mardan, Pakistan. *Int J Res Med Sci*, *20*(4), pp.7-11.

Simmonds, P., 1995. Variability of hepatitis C virus. Hepatology, 21(2), pp.570-583.

Thompson, M.E. and Barkhuizen, A., 2003. Fibromyalgia, hepatitis C infection, and the cytokine connection. Current pain and headache reports, 7(5), pp.342-347.

Waqas, M., Parveen, F., Waqar, M., Ali, A., Khan, M.A., Wasim, M., Khan, A.U., Rehman, Z., Ahmad, T., Shereen, M.A. and Nawaz, R., 2015. Distribution pattern of various genotypes of HCV circulating in district Mardan, Khyber Pakhtunkhwa, Pakistan. *Advan Biol Res*, *9*(2), pp.69-74.

Weigand, K., Stremmel, W. and Encke, J., 2007. Treatment of hepatitis C virus infection. World Journal of Gastroenterology: WJG, 13(13), p.1897.

Wiley, T.E., McCarthy, M., Breidi, L., McCarthy, M. and Layden, T.J., 1998. Impact of alcohol on the histological and clinical progression of hepatitis C infection. Hepatology, 28(3), pp.805-809.

Yen, T., Keeffe, E.B. and Ahmed, A., 2003. The epidemiology of hepatitis C virus infection. Journal of clinical gastroenterology, 36(1), pp.47-53.

Zeeshan, M. and Shah, M., 2019. Chemiluminescent microparticle immunoassay-based detection on blood donor and incidence of HCV virus in district Mardan Pakistan.

Zein, N.N., 2000. Clinical significance of hepatitis C virus genotypes. Clinical microbiology reviews, 13(2), pp.223-235.