CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Prevalence of Hepatitis B and C in General Population and Transgender Population of Rawalpindi

by

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A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2021

Dedicated to ALLAH Almighty, Hazrat Muhammad (PBUH) & my Parents.

CERTIFICATE OF APPROVAL

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Acknowledgement

I humbly thanks to **Allah Almighty**, the Merciful and most Beneficent who best owed his innumerable blessings upon mankind, one of which is knowledge a distinction for mankind. I offer my gratitude to the Holy Prophet Muhammad (PBUH) who preached us to seek knowledge for the betterment of mankind in particular and other creatures in general.

I am deeply indebted to my supervisor **Dr. Erum Dilshad**, Assistent Professor, Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology Islamabad (CUST), Pakistan. Her guidance, continuous encouragement and productive criticism throughout my study have helped me in completion of my thesis. Finally, to the most special persons I have in my life, my family, who have always present for my help. I am thankful for every moment.

Thanks to all.

(Hasnain Waheed)

Abstract

Hepatitis B and C infections are global health problem, and both cause acute and chronic infections in human. Hepatitis B and C both damage the liver and cause cirrhosis and hepatocellular carcinoma. Pakistan is among those countries where the rate of hepatitis B and C infections is much high and it is estimated that 15 million people are infected with hepatitis in Pakistan. The main reason of high infection rate in Pakistan is unawareness and lack of screening facility. In this study, we found the prevalence of hepatitis B and C in general population and in transgender population of Rawalpindi city. We also discussed the different biochemical parameters of hepatitis patients. The screening of general population and transgender population was conducted in different regions of Rawalpindi city. HBsAg rapid test card and rapid anti-HCV test card were used for the screening of hepatitis B and C. Different biochemical parameters of hepatitis B and C patients were also studied. Chi-square test was performed to find out the correlation between different factors. In general population, 98% people were negative to hepatitis B virus while 2% showed positive result. For hepatitis C virus, 10.6% people were infected with HCV while 89.3% were negative to HCV. In transgender population, 8% transgenders were positive to hepatitis b virus while 92% transgenders were negative to HBV. For hepatitis C virus, 14% transgenders were positive to HCV while 86% transgenders were negative to HCV. Hepatitis B and C were common in males than females. Hepatitis B and C were also common in people of age more than 40 years. Most of the hepatitis patients had blood group A and B while most of them had high hemoglobin level than 12g/dL. Most of the HBV and HCV patients showed elevated level of ALT. For hepatitis B, most of the patients were infected with genotype B, C and D while no one was infected with genotype A, E, F, G and H. Most common genotype for HBV was genotype D. For hepatitis C, most of the patients were infected with genotype 1, 2 and 3 there was no one infected with genotype 4, 5 and 6. Most common genotype for HCV was genotype 3. To find out the correlation between age and genotype of HBV Chi-square test was performed the P-value was 0.000 that is less than 0.05 that indicate our data was significant. Correlation for HCV genotypes and age indicate the P-value is 0.487

that means the data was not significant. Also, to find the correlation between gender and genotypes of HBV the P-value is 0.007 that is less than the standard value of 0.05 that indicates our result was significant. In order to find out the correlation between gender and genotypes of HCV the P-value was 0.006 that is also less than from the standard value of 0.05 indicates that result was significant. In general population, the prevalence of hepatitis C was more than the hepatitis B. In transgender population, the prevalence for hepatitis C was also more than the hepatitis B. Results show that males were more infected with hepatitis B and C than females. Most of the HBV and HCV patients had age more than 40 years. Most of the HBV and HCV patients had hemoglobin level more than 12g/dL. In Rawalpindi, most common genotype of hepatitis B virus is genotype D and most common genotypes for hepatitis C virus is genotype 3.

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Abbreviations

CDC	Center for Disease Control
cccDNA	Covalently Closed Circular DNA
GHSS	Global Health Sector Strategy
HIV	Human Immunodeficiency virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HbsAg	Hepatitis B surface Antigen
IDU's	Injecting Drug Users
NHSF	National Hepatitis Strategic Framework
SDG's	Sustainable Development Goals
STD's	Sexually Transmitted Diseases
UNO	United Nations Organization
WHA	World Hepatitis Alliance
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Hepatitis B and C infections are global health problem, and both cause chronic and acute infection in human. Hepatitis B and C virus both damage the liver. According to World Health Organization (W H O, 2019), It is estimated that about 325 million people worldwide are affected with hepatitis and about 1.4 million deaths annually are due to viral hepatitis B and C. After tuberculosis, hepatitis is the second most powerful killer infection and as compared to HIV people are 9 times more infected with hepatitis [1].

The DNA of hepatitis B virus is double stranded and belongs to the family Hepadnaviridae. This virus causes disease hepatitis B in human. Hepatitis B virus infects liver and if not treated the infection turns to chronic stages and then hepatocellular carcinoma and cirhosis are developed. The DNA of hepatitis B virus contains some unusual features that are similar to retroviruses. Hepatitis B virus have 10 genotypes that ranges from A to J [2]. The HBV virion is 42 nm in diameter, spherical double shelled structure. The outer envelope is made up of lipid and HBsAg surrounds nucleocapsid that is made up of hepatitis B core antigen. The nucleocapsid contains viral DNA genome and virally encoded polymerase. The HBV genome size is 3.2 kilobase pairs and containing double stranded DNA

[2]. The lifecycle of HBV starts when virion attach specific receptor. After the attachment of viral receptors with cell membrane receptors releases nucleocapsid into the cytosol of the cell. When the capsid is removed, the viral DNA along with DNA polymerase enzyme enter into the nucleus of the cell. The polymerase enzyme repaired the viral DNA and convert it into double stranded DNA which is undergoes supercoiling to form covalent closed circular DN(cccDNA). The viral DNA of HBV persisit in the cell in the form of cccDNA. The half-life of cccDNA is very long and it is not integrated into the cellular genome [3]. An acute hepatitis B infection occurs when a person first expose to hepatitis virus. In most of the cases healthy adults do not show any symptoms after exposure to hepatitis b virus and get rid of virus without any problem. But in some cases when the acute infection is not treated then it may convert into severe chronic conditions that are difficult to handle. Acute infection may last up to 6 months and infected person is able to infect others during this time. Low fever, bone and muscle pain, loss of appetite and usually stomach pain are the symptoms of acute infection. Proper symptoms do not show in some people, but some people face severe symptoms including vomiting, nausea and jaundice [4].

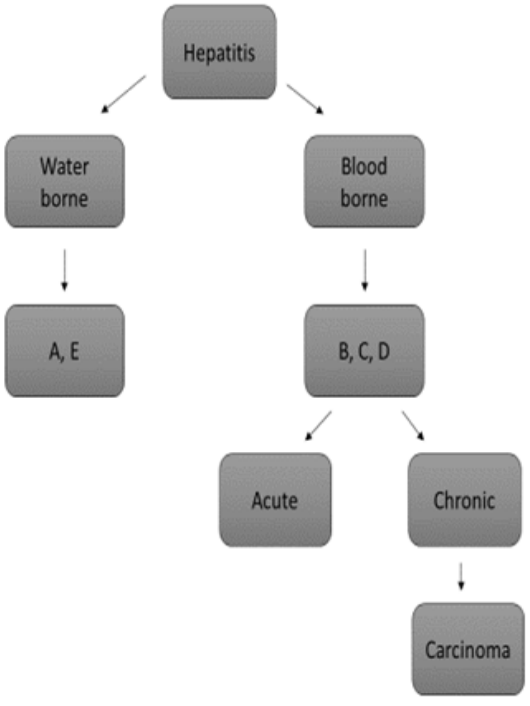

FIGURE 1.1: Types of Hepatitis and Infection Flow

There are several types of hepatitis that shows different effects on human body. Different viruses are responsible for different hepatitis type. Hepatitis A and E are usually waterborne disease and shows short term and acute infection. Hepatitis E sometime turns to dangerous in pregnant women. Hepatitis A is caused by hepatitis A virus (HAV) and transmitted by edible things like food, water and other edible infected by feces of Hepatitis A patient.

Hepatitis B is transmitted by body fluids like vaginal secretions, semen, and blood of hepatitis B patient or by sharing having razors, having sex and injecting drugs. Hepatitis C is most fatal viral disease and caused by hepatitis C virus (HCV) and its mode of transmission is as similar to hepatitis B. Different types of hepatitis can be seen in Figure 1.1.

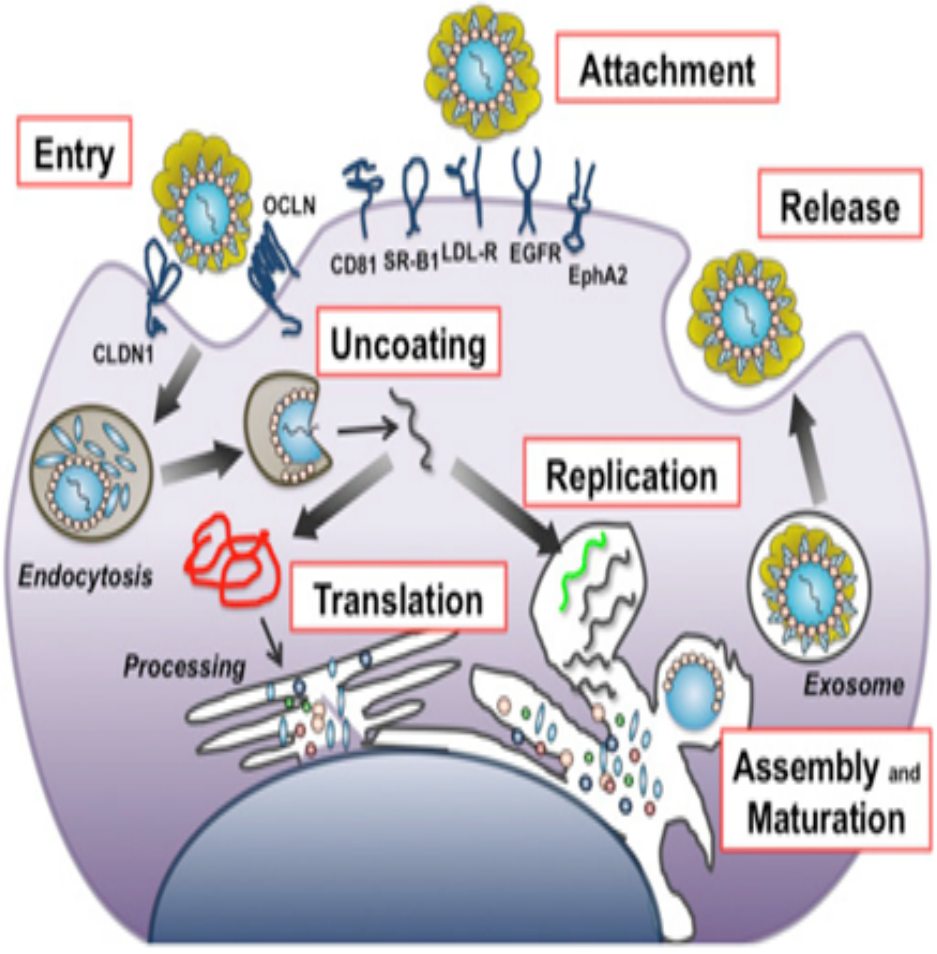

FIGURE 1.2: Replication Process of Hepatitis B virus [5]

The replication cycle of hepatitis C virus starts from the attachment of viral particles on the host cell surface receptors. After binding with receptors, the virus enters into the cell by endocytosis. After endocytosis the positive strand RNA genome release into the cytosol of the cell. The viral genome is replicated in host cell and form its multiple copies. After replication the next step is assembly and maturation, in this step the viral particles got maturated by developing capsid around the genome and now viral particles release outside the cell by exocytosis to infect other cells [5]. The process of HCV replication can be seen in Figure 1.2.

Chronic HBV infection happen when a person tests positive for more than six months. This means that their immune system is not able to get rid of infection and it still remain in the liver and blood. The chances of getting chronic HBV infection is depend on at which age person first exposed to hepatitis B virus. Most of the people diagnosed with hepatitis B virus in last stages and their body do not show any symptoms [4].

If chronic HBV infection is untreated for many years, it may turn into hepatocellular carcinoma and cirhosis. The sign and symptoms of hepatitis B infection depend upon status of infection that is acute or chronic. The acute infection sometime shows no or mild symptoms including low grade fever, bone and muscle pain and loss ap appetite. In case of chronic infection, the symptoms vary from normal to severe conditions.

About 15-20% of chronic patient develops severe liver disease hepatocellular carcinoma and cirhosis. Risk factors thar are responsible for hepatitis is much more similar to HIV. Hepatitis is caused by hepatitis B virus and spread from person to person in different ways. Sharing needle is a major factor to infect people.

If blood of infected person is expose to needle and that needle use by another healthy person so this person is at high risk of getting infection. Sharing needles during intravenous drug use is also a major cause for hepatitis B infection. The unprotected sex in which body fluid like semen, saliva and blood of infected person enter into a healthy person's body may cause an infection. A pregnant mother who is hepatitis B positive can pass it to their baby during childbirth. Transmission

of hepatitis B virus is also due to unprotected blood transfusion. If the blood of infected person is transferred to healthy person without proper screening may cause hepatitis B infection.

Hepatitis C is a type of infectious disease that is usually caused by hepatitis C virus and affects the liver. Hepatitis C is a RNA virus, small in size, RNA is single stranded and virus attack on liver cells [6]. The family od hepatitis C virus is Flaviviridae and this virus not only cause hepatitis but also involve in developing liver cancer, cirrhosis and lymphoma in human. During the initial stages of infection commonly no symptoms appear. But later on, during late chronic infection severe symptoms appear that damages 60-70% of the liver tissues.

1.2 Aims and Objectives

Hepatitis B and C are the common viral diseases that are commonly present in our society and mortality rate due to hepatitis b and c is very high but unfortunately most of the people are unaware about these diseases. The aim of this study is to find out the prevalence of hepatitis B and C in our population. The study contains following objectives.

- To find out the prevalence of Hepatitis B and Hepatitis C in general population of Rawalpindi.
- To find out the prevalence of Hepatitis B and Hepatitis C in transgender population of Rawalpindi.
- To determine the biochemical parameters of HBV and HCV positive patients from hospital?
- To perform genotyping of prevalent strains Hepatitis B and Hepatitis C?

Chapter 2

Literature Review

In 2018, Viejo and his co-workers performed hepatitis screening on 2637 participants of general population. Rapid test of anti-HCV antibody test was performed to check out the prevalence of hepatitis in this population.

The screening result of 30 participants was positive and 23 out of 30 had received antiviral treatment and aware about the positive status of their infection. In the same way, 7 participants out of 30 were unaware about their hepatitis infection [7].

Hepatitis is a silent disease for many years and screening is not common in the world that is why WHO forced to speed up the screening procedure to find out the missing millions.

Most of the people aware about their infection in later stages of disease when more than 70% of the liver tissues become damaged. The overall death rate due to hepatitis B and C is very high and hepatocellular carcinoma is the second leading cause of death in the world.

There are different sources that causes hepatitis B and C, different sources included sexual contacts, injecting drug use, blood transfusion without proper screening etc. Different sources for hepatitis b and c can be seen in Figure 2.1.

Hepatitis B and C risk factors are quite common and co-related with HIV and other sexually transmitted diseases. It is a very serious health issue as 325 million people yearly live with hepatitis.

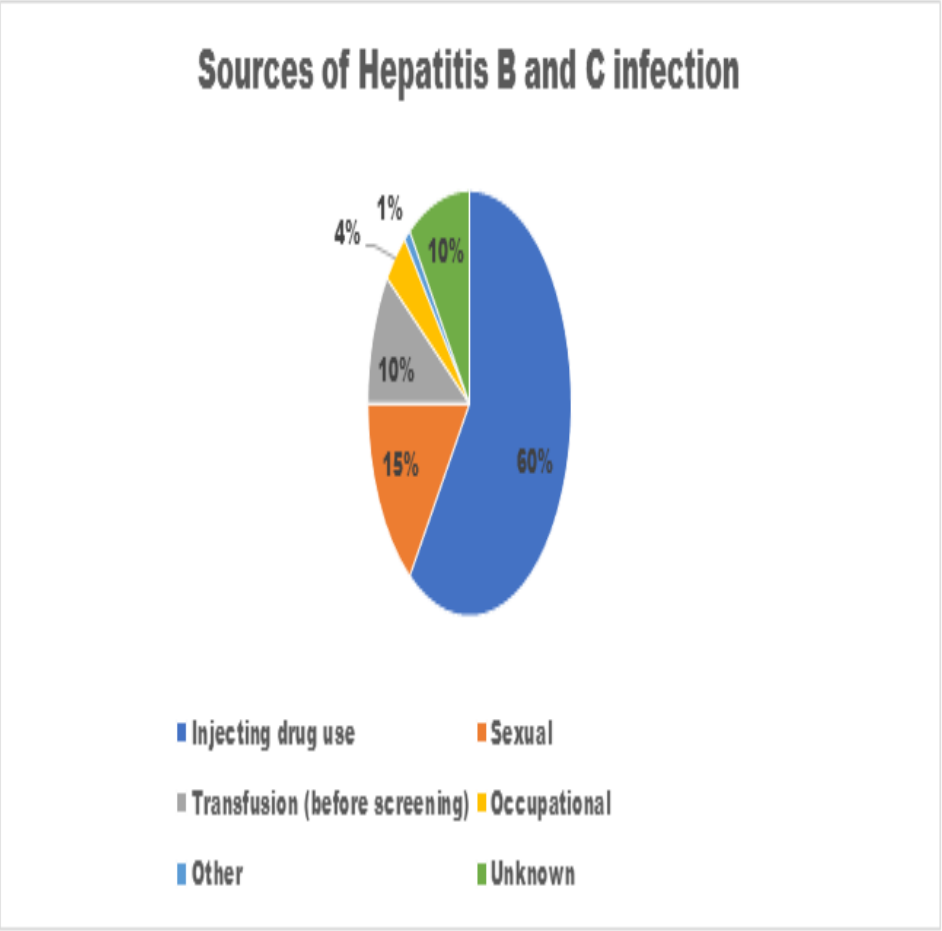

FIGURE 2.1: Major Sources of Hepatitis B and C Infection [8]

Acute infection shows mild symptoms sometime and people easily catch this virus from their surroundings such as from health care workers, barbers, by nose and ear piercing, tattooing and accidental syringe prick. According to Center for Disease Control (CDC), major sources for hepatitis B and C transmission is injecting drug use and sexual contacts [8].

According to World Hepatitis Alliance (WHA), about 290 million people living with hepatitis, but they are unaware about their infection. Without screening and diagnosing, millions of people get infected and thousands will lost their lives [9].

In advanced countries where all health facilities are available, they also lack in screening of general population to find out the missing millions. In developing countries like Pakistan, where no proper health facilities are available and due to low literacy rate and unawareness the number of hepatitis infection increases day by day.

2.1 Acute and Chronic Stages of Hepatitis C

Different stages of hepatitis C infection which depends upon the duration of viral entry in the body. At very early stage, when HCV enters in the body it shows little or no symptoms but after the passage of time the viral particles replicate more in the hepatocyte cells of liver. Mild symptoms appear like low grade fever, loss of appetite, joint pain and abdominal pain etc these are the sign of acute HCV infection. Acute HCV infection may last up to six months after the exposure to virus. But if the HCV test result positive after 1-2 years of infection, this is the sign of chronic HCV infection.

This means the virus is active in the blood stream and continuously replicate within the hepatocyte cells of liver and may shrink the liver. Chronic HCV infection is fatal when untreated severe symptoms appear like nausea, high fever, vomiting and jaundice. If chronic stage is untreated the virus may infect 70-80% of the liver tissues and later may develop hepatocellular carcinoma and cirrhosis. The causes and risk factors for HCV infection is much more similar to HBV infection as well as HIV infection. Blood and blood products play important role in transmission of hepatitis in human. Ear and nose piercing, Tattooing, reuse of syringes are also a main cause of viral transmission in general public.

2.2 Global Burden of HBV

Hepatitis B disease is a global public health issue that is faced by world for many years. Every year about 30 million people are infected with hepatitis B virus.

About 8.5 million people die every year with HBV infection and other diseases of liver. According to World Health Organization (WHO), about 325 million people globally live with hepatitis. About 2.6 million people infected with HIV are also co-infected with HBV [10].

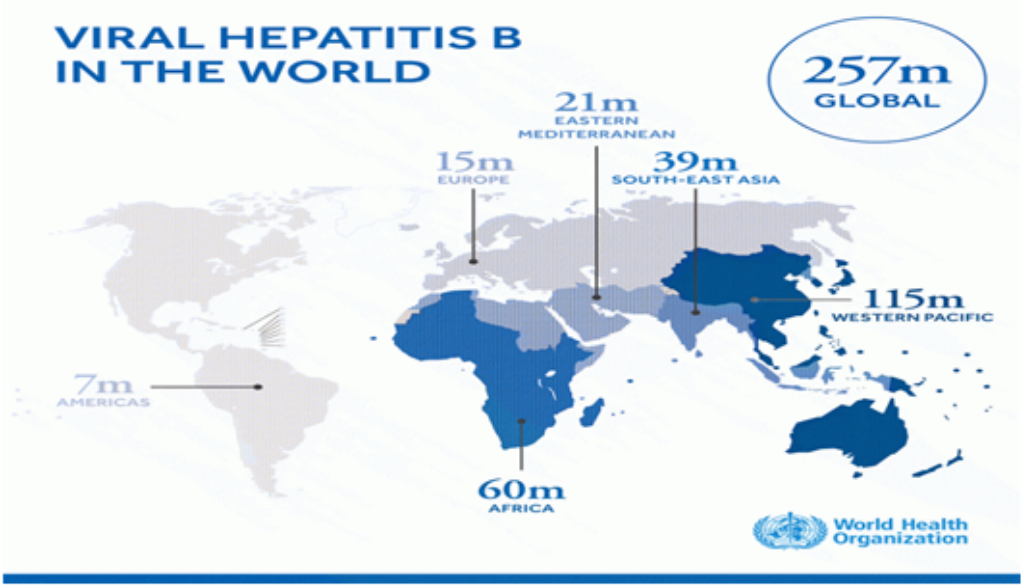

FIGURE 2.2: Global Distribution of Hepatitis B Virus [11]

HBV is a global health issue and about 257 million people around the globe are affected with hepatitis b. In western pacific region 115 million people are affected with HBV. The details of other region of world affected with HBV is discussed in Figure 2.2. HBV is approximately 100 times more infectious as compared to HIV. It is the main cause of liver cancer, which is the second leading cause of death in the world. In 2015, Sustainable Development Goals also included elimination of hepatitis by 2030. Global Health Sector Strategy on Viral Hepatitis passed by The World Health Assembly in 2016, to eliminate hepatitis by 2030 [7].

2.3 Global Burden of HCV

Hepatitis C disease is caused by Hepatitis C virus that is bloodborne. It is a serious health issue worldwide face by human. According to World Health Organization

(WHO) there is about 325 million people living with hepatitis globally and there is about 71 million people have chronic HCV infection. The highest number of chronic HCV infection (15 million) is reported in Eastern Mediterranean region. Details of chronic HCV infection in different region of the world represented in Figure 2.3. About 1.4 million deaths per year worldwide are due to acute HCV infection and hepatocellular carcinoma and cirrhosis. From these deaths about 47% deaths are due to HBV and 48% are due to HCV infection. It is estimated that 2.9 million people living with HIV are also infected with hepatitis C virus. It is about 67% of injecting drug user are infected with hepatitis C virus. Most of the HCV infected people are living in north and west Africa and central and east Asia due to unsafe medical procedure and injections [12].

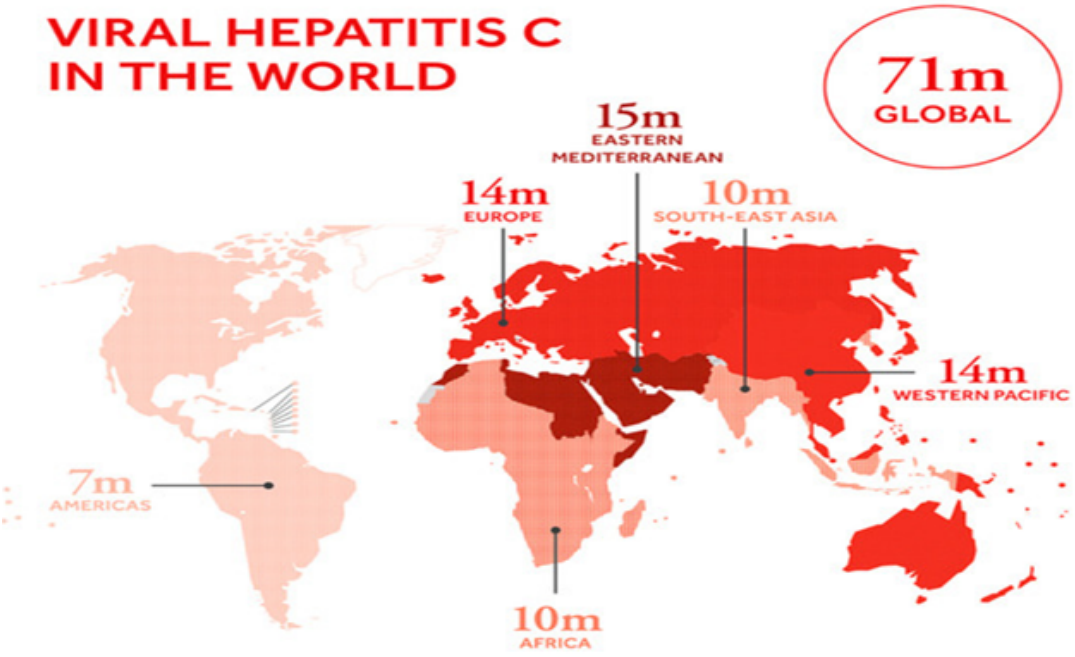

FIGURE 2.3: Global Distribution of Hepatitis C Virus [13].

2.4 Virology of Hepatitis C Virus

Hepatitis C virus is a single positive stranded RNA virus and its genome contains about 9400 bases. According to genome organization and method of replication

HCV belongs to the family Flaviviridae [14]. HCV particles are 40-70 nm in diameter that contains single stranded RNA genome and contains E1 and E2 as core and envelope glycoproteins. The nucleocapsid is form when HCV genome interact with core protein and this nucleocapsid is surrounded by a membrane made up of lipid called as viral envelope. So, the glycoproteins that are attached on the viral envelope. The HCV glycoproteins located on viral envelope are also responsible for HCV entry into the cell. These glycoproteins attached on the receptors and mediate the fusion process between the host cell membrane and viral envelope. HCV glycoproteins E1 and E2 are belong to type 1 transmembrane proteins [15].

2.5 Genotypes

Hepatitis B virus is the common virus of human and present in all over the world. In general population of different countries the rate of HBV infection is 2% to 20% [16]. It is estimated that 15%-40% of hepatitis B carriers have high chances of liver failure, hepatocellular carcinoma and cirrhosis. Hepatitis B virus is the smallest human DNA virus that contain 3200 base pairs. Reverse transcriptase enzyme transcribed pre-genomic RNA from covalently closed circular DNA (cccDNA). This unique replication strategy makes it to evolve more genotypes and sub-type mutants. Hepatitis C virus contain eleven genotypes and six are the major genotypes. These genotypes are further divided into subtypes. The six HCV genotypes ranges from 1 to 6 and subdivided as 1a, 1b, 1c, 2a, 2b, 3a, 3b, 3c, 4, 5, 6. Globally different HCV genotypes are present in different regions.

2.6 Distribution of HBV Genotypes

HBV have almost 10 different genotypes (A to J) and several sub-types have been identified. Genotype A is very common Europe, America, India and Africa. Genotype C and B are very common in Asian region. Genotype D is common

in African region. Genotype E is very common in Western Africa. Genotype F is commonly found in South and Central America. Genotype G is present in Germany, France and some American regions. Genotype H is prevalent in Mexico and Central America. Genotype I was found in Laos and Vietnam and the newest HBV genotype J is commonly present in Japan [17]. Most common HBV genotype in Pakistani population is Genotype D. Different studies conducted in different region of Pakistan reported that Genotype D of HBV is most commonly present with rate of prevalence is 63.71%, Genotype A prevalence rate 10.036%, Genotype C is 7.55% and Genotype B is 5.335% while mixed and untypable genotypes were 9.931% and 2.377% respectively [18]. In Asian region genotype B and C are very common in people and different region of the world have different genotypes as represented in Figure 2.4.

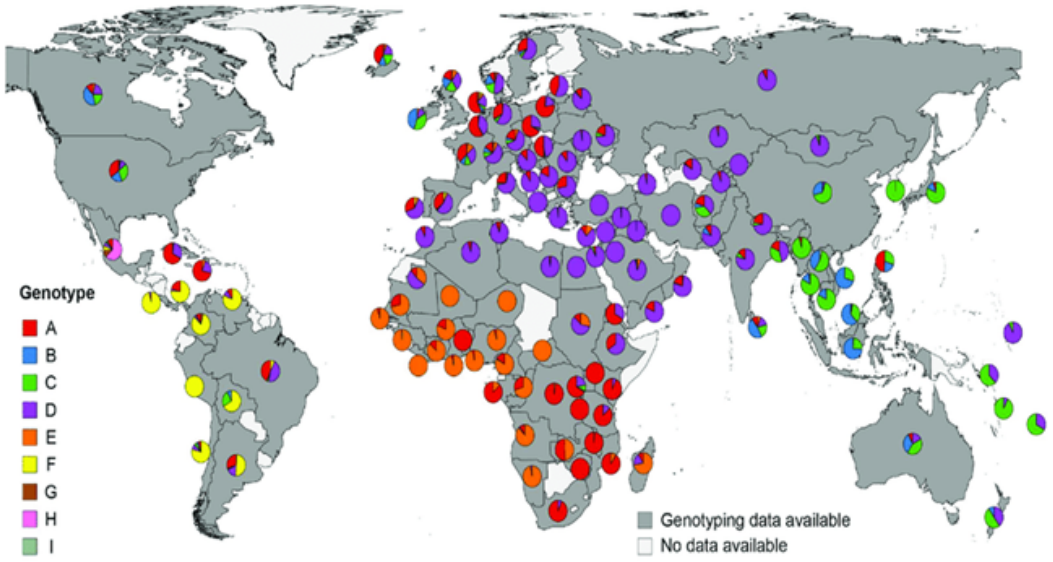

FIGURE 2.4: Global Distribution of Hepatitis B Virus Genotypes [19].

2.7 Distribution of HCV Genotypes

HCV is divided into eleven different genotypes six out of eleven are major genotypes that are further divided into subtypes. Around the globe the most predominant genotypes of HCV are 1 and 3. In African region the most commonly

HCV genotype is 4 with a high proportion of genotype 1 and 2. In Middle east and North African countries HCV genotype 4 is predominantly occur while some also infected with genotype 1. In America the HCV genotype 1 is most common among the people. In Asian countries, HCV genotype 1 is most commonly found along with genotype 3. In Australia, most common genotype of HCV is genotype 1. In European countries, HCV genotype 1 is commonly present in all the countries along with some cases of HCV genotype 3. In Pakistan, the most common genotype of HCV is genotype 3. According to the data, 78.96% of people infected with HCV genotype 3, 7.04% people infected with HCV genotype 1 and only 5% of people infected with mixed genotypes. It is about 3.82% people have HCV genotype 2. In the same way, 3.30 people infected with unstoppable genotype and less than 2% of people contain genotype 4, 5 and 6 [16]. There are different HCV genotypes around the globe as represented in Figure 2.5. Form this data it is cleared that HCV genotype 3 is commonly present in Pakistani population. The HCV genotype 3 is usually easy to treat as compared to HCV genotype 1 and it also have low side effects than other genotypes.

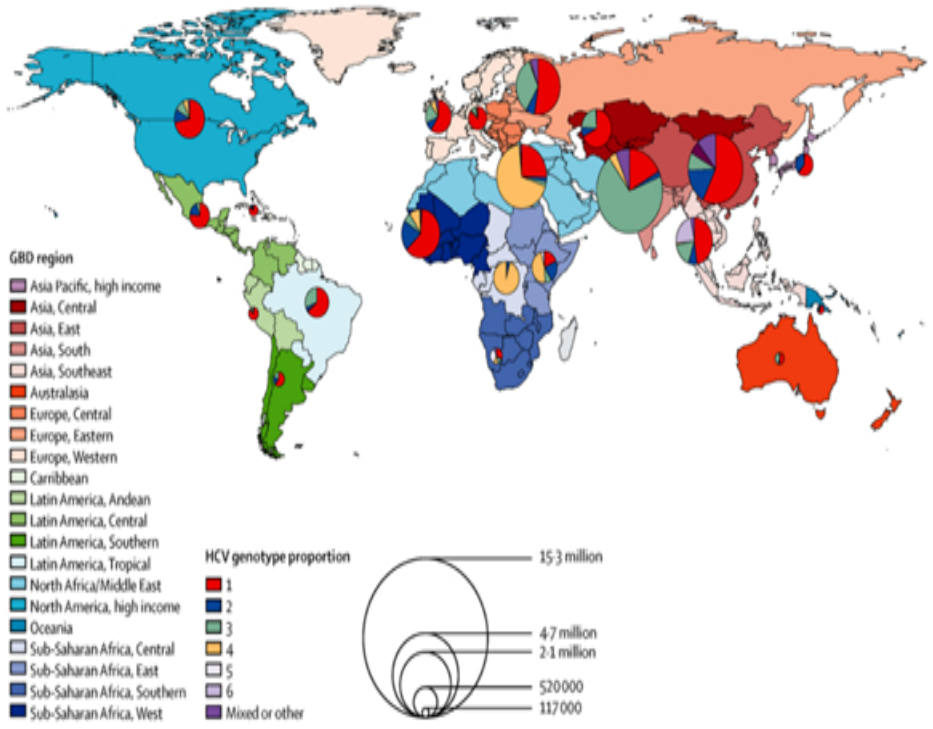

FIGURE 2.5: Global Distribution of HCV Genotypes [20].

2.8 Status of Hepatitis in Pakistan

According to WHO, in Pakistan 15 million people are infected with hepatitis. Thousands of new patients are infected every year due to unawareness of viral transmission. In Pakistan lack of prevention and treatment resources are the main reasons of viral transmission. Unsterilized medical instruments, unscreened blood transfusion and unsafe injections are the reasons for increasing infection rate day by day [21]. There are 170 million people in Pakistan with low health and education standard. There is no proper awareness program by the government to educate the people about the transmission of viral hepatitis. Unsterilized medical instruments and unprofessional medical staff is responsible for the transmission of hepatitis. Barbers community in Pakistan is also involve in transmission of viral infections. They use unsterilized instruments and potash alum on every person increasing the risk of viral transmission. Some barbers in different region of country also uses permanent razors that is the basic reason of the transmission of blood-borne infection. High rate of hepatitis infection in Pakistan is due to negligence of screening people. So, Government of Pakistan should boost screening procedure to find out the missing millions that are living with hepatitis but they don't know. Screening of general population as well as high risk population is the basic step to find out the incidence of hepatitis infection in Pakistan.

2.9 High Risk Populations of Pakistan

Hepatitis B and C is very common in Pakistani population but most of the people are unaware about the infection. Hepatitis is commonly transmitting through infected blood or blood products and sexual contacts. In Pakistan, different communities are at high risk for the transmission of hepatitis b and c such as transgenders, barbers, injecting drug users (IDU's) and health care workers. Transgenders are well known for their sexual activities that is why they are at the top of high-risk population. Sexually transmitted diseases are very common in transgender population and in this way the infections easily transmitted to general public.

Barbers are also involved in the transmission of infectious disease due to their occupation. Most of the barbers are uneducated and they do not know how much contaminated blood is dangerous. Barbers usually use unsterilized equipment on every client and some barbers uses permanent razor on most of the client in this way they involve in the viral transmission. Some barbers traditionally use potash alum on every client for skin cuts for healing, this potash alum is also involved in transmission of viral diseases. Barbers usually rubbing same potash alum on facial cuts of different customers [22]. High rate of infectious diseases are present in injecting drug users (IDU's) community. IDU's are illiterate as well as low standard of living in the society. They spend most of their earning on buying drugs. They usually inject drug intravenously and most of the time one syringe is used by multiple people. They do not follow any precautionary measures. They are using one syringe of drug in multiple people that is why they have high rate of infectious diseases. Health care workers are also at high risk of developing hepatitis because of their multiple time exposure to blood-products and blood. The accidental needle stick might be serious sometime and can be enough to develop HBV or HCV infection [23].

2.10 Transgender as a High Risk Population in Pakistan

Transgender community of Pakistan is at high risk for sexually transmitted diseases. They often lack social support and are at high risk for substance use, commercial sex works, unemployment, homelessness which increases their risk for developing infectious diseases. Due to the lack of social support of transgender there is no proper screening data available yet.

Some transgenders usually live their lives by begging but most of the community members are sex workers due to unemployment. Government do not start any campaign to proper screen out the whole transgender population. Some transgenders are also addict of drugs and usually take intravenous drugs. There is no

proper screening data available yet that is why we predict a huge percentage of infectious diseases in transgenders due to their social behaviors.

2.11 Sustainable Development Program

For last many years, a big health threat (hepatitis) is being ignored internationally but recently in 2015, United Nations Organization (UNO) introduced Sustainable Development Goals (SDG) to protect planet. WHO introduced 17 life changing goals to protect and help the humanity worldwide.

Elimination of viral hepatitis is lying in goal 3 (Good Health and Well-being) of SDG. WHO starts a program to eliminate hepatitis by 2030, many of the developed countries are on the right track but unfortunately underdeveloped countries are too far a way to achieve these goals.

2.12 Finding the Missing Millions

The World Hepatitis Alliance (WHA) starts a campaign named as Finding the Missing Millions a three-year global awareness campaign to screen out and diagnose the general public for hepatitis. This campaign totally focuses on some aspects such as providing knowledge of disease to general public and providing easily accessible testing kits to screen out the population [24].

Find the Missing Millions is a three-year global awareness-raising and advocacy campaign aimed at tackling the main barriers to diagnosis by putting civil society organisations and the affected community at the heart of the solution.

In Pakistan, there is no proper screening facility available for general public that is why the infection rate of hepatitis is very high in our country. Most of the people living in Pakistan is unaware about their health status and don't try to test himself without any problem. Screening is the first step of finding missing millions so big effort requires in this sector.

2.13 Hepatitis Elimination by 2030

After the introduction of hepatitis in 17 SDG's by United Nation Organization (UNO) the WHO developed Global Health Sector Strategy (GHSS) to eliminate hepatitis by 2030. There are various methods in GHSS that will take part in lowering the rate of incidence of hepatitis by 2030. In November 2017, about 84 countries developed their strategies and national plans to control hepatitis [25]. Other nine developed countries are also on the right track to achieve hepatitis elimination by 2030 but this goal is un-achievable for underdeveloped countries due to lack of resources [26].

2.14 Hepatitis Elimination and Pakistan

WHO working with health authorities of different countries to speed up their hepatitis control programs in order to achieve the elimination of hepatitis by 2030. The screening is the initial step to find out the missing millions living in our society that are usually unaware about their health status. In October 2017, Government of Pakistan had launched the National Hepatitis Strategic Framework (NHSF) (2016-2021) to elaborate the contribution of health sectors in combating with viral hepatitis. The main theme of NHSF is to provide affordable, easy approach, effective prevention and treatment services to people living with hepatitis infection [27]. The main routes for the transmission of hepatitis in Pakistani population are reuse of needle and syringes, shaving from barbers, unscreened blood transfusion and reuse of same surgical and dental instruments on different patients.

Chapter 3

Methodology

The cross-sectional study conducted in different areas of Rawalpindi city to find out the prevalence of hepatitis B and C in general population as well as in transgender population of Rawalpindi city.

A data acquisition form was design to collect the demographic and socio-economic data and risk factors of hepatitis B and C including age, gender and hepatitis history. Data was collected from general population and transgender population of different regions of Rawalpindi city.

3.1 Sample Collection

The random samples of general population were collected from different regions of Rawalpindi city. The samples were collected from people above the age of 18 including male and female both. Total of 150 samples were collected from general population and 150 samples of transgender population.

The samples were collected from the lower-class area, middle-class area and upper-class area of Rawalpindi city. The blood samples taken from the suitable site of venipuncture to collect the blood. The blood samples were collected and placed in a container with proper labelling of name and serial number of participants.

3.2 Demographic Information

Demographic information's include personal details of subjects which includes age, gender, family history, life-style, diet habits and taking any antiviral medication in the past.

3.3 Blood Sampling

Each person was asked to sit relaxed, suitable site for venipuncture to collect blood by placing the tourniquet 3 to 4 inches above was selected for insertion of syringe on the subject arm or back side of hand. Vein was selected, cleaned in a circular motion, after the area was cleaned it was touched or palpated again. Person's arm was firmly gripped using thumb to draw the skin stretched and anchor the vein. Needle was inserted into the lumen of vein at an angle of 15-30 degree with the arm surface. Syringe was filled for 5CC blood.

Tourniquet was removed first and then needle removed from the person's arm by applying backward motion. Alcohol swab was placed immediately on the puncture site of the arm and adequate pressure apply on the puncture site to avoid the formation of hematoma. After holding pressure for 1-2 minutes 5 ml blood collected and placed in two different containers with proper labelling. 3 ml blood was used for hepatitis C test kit and 2 ml blood was used for hepatitis B test kit.

3.4 Rapid Test Kits for Hepatitis B and C

A rapid test kit was used for the detection of hepatitis B and hepatitis C in general population and transgender population as seen in Figure 3.1. Rapid test kits are very effective in giving accurate result within 5 minutes. The two drops of blood added in sample adding area of the kit. After adding sample, buffer solution added and now sample was run with capillary action and gave result by the formation of bands.

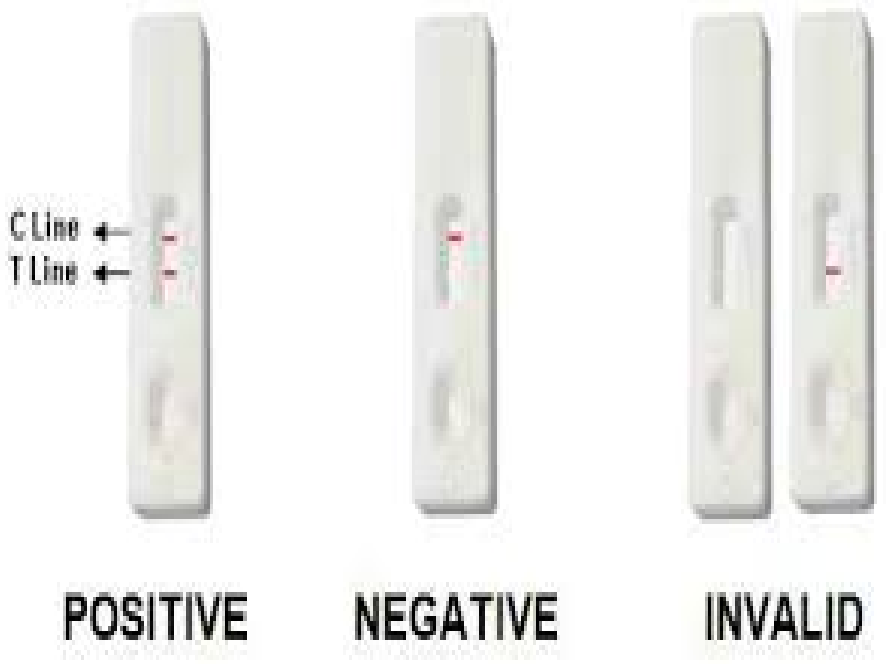

FIGURE 3.1: How Hepatitis B and C Test Performed on Rapid Test Kit [28]

3.4.1 HBs Ag Detection by Rapid Card Test

Hepatitis B surface antigen test device is a rapid test technique usually used to detect the presence of HBV antigen in blood. The rapid test device is coated with anti-HBsAg antibodies on the test region of the device.

During test the sample is loaded on the sample adding point and the sample is run on the device with capillary action. The chromatographically migrated sample gives colour line on the test and control region. There are two regions on the membrane one is control region (C- region) and other is test region (T- region) as shows in Figure 3.1.

If the colour line appeared on both control region and test region, it indicates that test result is positive. If the colour line is appear on control region and absence of colour line on test region indicates that test result is negative. If there is no colour line appeared on control region and test region, it indicates that the appropriate amount of sample is not added.

3.4.2 Rapid Anti-HCV Rapid Card Test

A rapid Anti-HCV test is a immunochromatographic assay for the detection of antibodies of hepatitis C virus in human blood. The membrane of device is coated with hcv antigen on the test line region present on device. The 2 drops of blood are added on sample adding point along with 1 drop of buffer solution is reacting with membrane coated hcv antigen. The sample mixture is moving upward on the membrane with the help of capillary action.

The migration of sample reacts with hcv antigen and produces colour line on the membrane of the device. There are two regions on the membrane of device one is control region (C- region) and second is test region (T- region). The appeared colour lines will indicate the result of test. If the colour line appears on control and test region this will indicate that the result is positive.

If the colour line appears only on control line and there is no colour line shown on test line this will indicate that test is negative. The sensitivity of Advanced quality rapid anti-HCV kit is 98.56%.

Serological assays play a significant role for the detection of hepatitis B and C virus. These assays are based on the host immune response and works on the principle of antigen-antibody interaction. Some kits are pre-coated with antigen and bind with the specific antibody present in the testing sample while some kits are pre-coated with antibodies and can be able to bind with the specific antigen present in the blood sample

3.5 Data Collection of Hepatitis Patients from Hospital

After performing screening of hepatitis B and C in general population and in transgender population, the data of hepatitis patients was collected from the Hospital. Many hepatitis patients are now properly treated in different hospitals of

Rawalpindi city. The data acquisition form was filled by collecting general information and health status of the patients.

The general information included name, age, gender while health information including blood group, hemoglobin level and genotype of hepatitis B and C virus. This information was very useful to find out the variations of different bio-chemical parameters in hepatitis B and C patients.

3.6 Biochemical Analysis

The biochemical analysis was performed to identify any deviations from standard level indicating the presence of disease. The biochemical analysis was used to find out the presence of antibodies of HBV and HCV in the blood.

The presence of antibodies indicate that the virus is present in the body and body develops antibodies against virus.

These antibodies fight against the virus and circulate in the blood. All the data and results were recorded on excel sheet. Biochemical analysis was performed by extracting blood and using immunochromatographic assay. Figure 3.1 summarizes the process biochemical tests.

3.7 Statistical Analysis

Prevalence of hepatitis B and C was obtained in the form of percentage by using MS Excel, Pie charts and bar charts are plotted respectively. Results are presented in the form of percentage.

For comparison purposes Chi-square test was performed and probability value (P-value) also determined. Significance is defined as when P-value is less than 0.05 and non-significant results obtained when P-value is greater than 0.05.

FIGURE 3.2: Snaps Taken During Sample and Data Collection

Chapter 4

Results and Discussions

4.1 Prevalence of Hepatitis B in General population

To find out the prevalence of hepatitis B in general population 150 individuals took part in screening. 3 individuals were identified with hepatitis B virus while 147 individuals showed negative results of hepatitis B viral infection.

The prevalence of HBV in general population was found 2%. Results are discussed in Table 4.1 and Figure 4.1.

According to Polaris Observatory the prevalence of hepatitis B in Pakistani population is 2.5% and our results of prevalence is 2% [29]. These findings show that our results are closer to Polaris Observatory data.

This data only belongs to the general population of Pakistan. According to Ali et al the prevalence rate for hepatitis B infection is 4.3% in Pakistan.

In the same way, the prevalence rate of HBV in healthy blood donors is 3.9% in Pakistan. The HBV prevalence rate is high in prisoners of about 5.3% [30]. In general population the rate of HBV infection is low as compared to blood donors, prisoners and transgenders.

TABLE 4.1: Frequencies of Healthy and HBV Infected Individuals.

	Frequency	Percent	Valid percent
Negative	147	98%	98%
Positive	3	2%	2%
Total	150	100%	100%

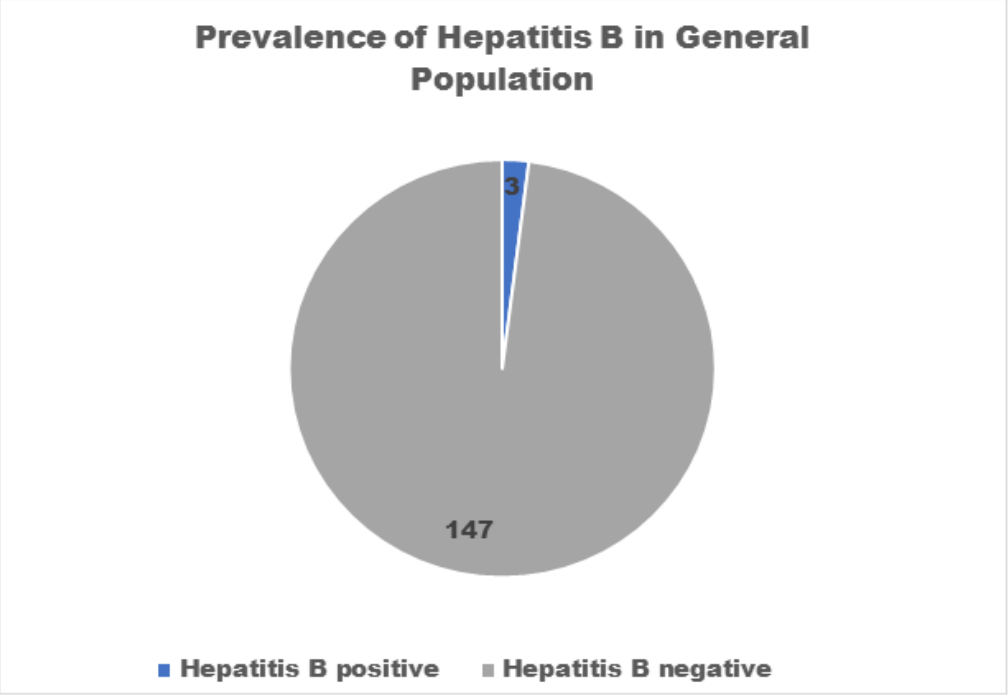

FIGURE 4.1: Prevalence of Hepatitis B in General Population.

4.1.1 Prevalence of Hepatitis C in General population

To find out the prevalence of hepatitis C virus in general population 150 individuals took part in screening camp. 16 individuals were identified with hepatitis c infection while 134 individuals showed negative results of hepatitis C viral infection. Overall prevalence of HCV in general population is 10.6%. Table 4.2 and Figure 4.2 summarized the results. According to Polaris Observatory 2015, prevalence of HCV in general population of Pakistan is 3.8% [31]. But now the

prevalence rate for HCV is much high in general population. Our findings indicate that 10.6% people are infected with HCV. The rate of positive HCV people is very high in rural areas of Pakistan. According to Khan (2018), the prevalence of hepatitis C in swat region of Pakistan is 5.2% that is very low to our results [32]. This indicates that the prevalence of HCV in general population of Rawalpindi is more than the general population of Swat.

TABLE 4.2: Frequencies of Healthy and HCV Infected Individuals.

	Frequency	**Percent**	**Valid percent**
Positive	16	10.6%	10.6%
Negative	134	89.3%	89.3%
Total	150	100%	100%

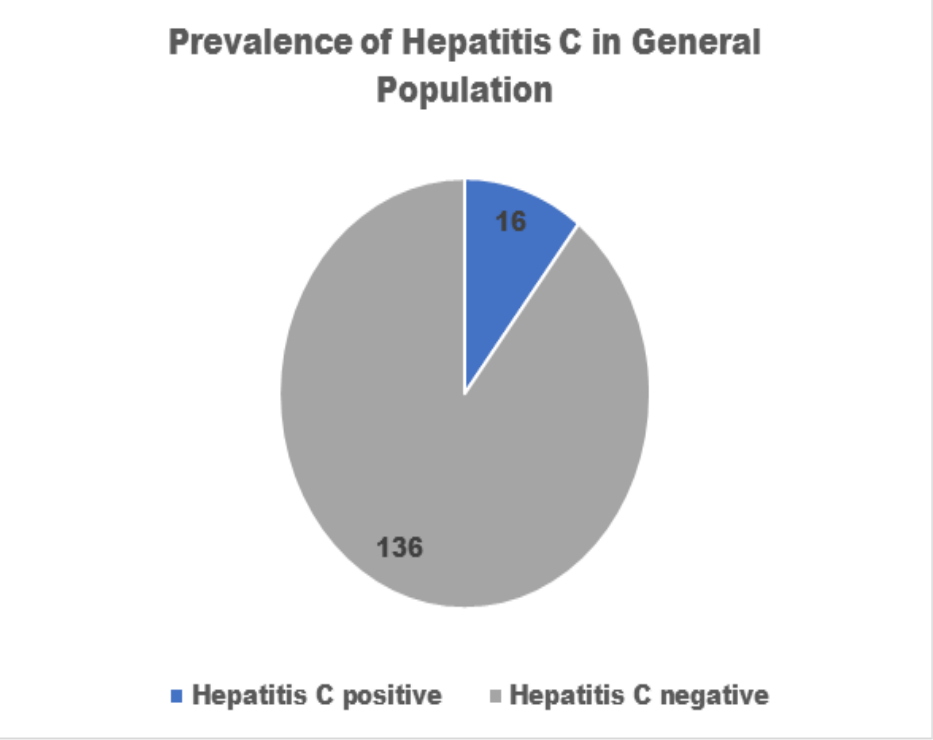

FIGURE 4.2: Prevalence of Hepatitis C in General Population.

4.1.2 Prevalence of Hepatitis B in Transgender population

To find out the prevalence of hepatitis B in transgender population, screening of 150 transgenders have been done, 12 transgender shows HBV positive result while 138 transgenders are negative for hepatitis b virus. The overall prevalence for HBV in transgender population is 8%. Results are described in Table 4.3 and Figure 4.3. The rate of HBV prevalence is high in transgender population as compared to general population. Transgender population are at high risk of developing sexually transmitted diseases (STD) due to their social behavior. High prevalence of HBV in transgender population indicates that they also have high rate of other sexually transmitted diseases. So, maximum screening is needed in this high-risk population. According to study conducted by Hadikusumo et al, 9.3% transgenders are positive to hepatitis B virus in Indonesia. High rate of HIV and HBV in transgenders living in Indonesia [33]. Transgenders are at risk of developing sexually transmitted diseases (STD).

TABLE 4.3: Prevalence of Hepatitis B in Transgender Population.

	Frequency	**Percent**	**Valid percent**
HBV Positive	12	8%	8%
HBV Negative	138	92%	92%
Total	150	100%	100%

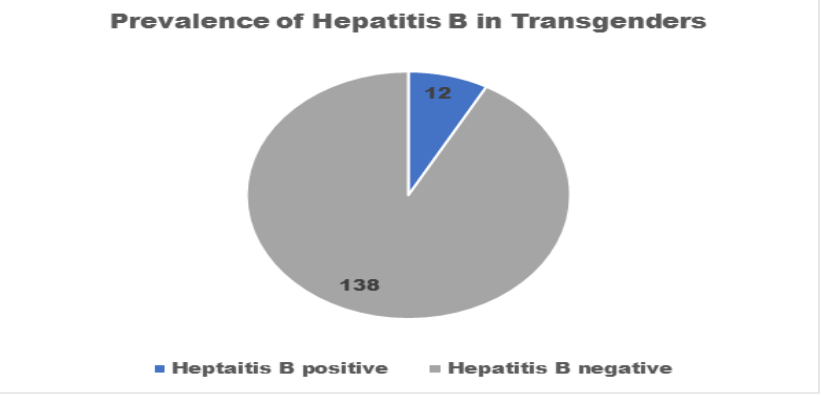

FIGURE 4.3: Prevalence of Hepatitis B in Transgender Population.

Prevalence of hepatitis B in transgender population results 8 percent positive and 92 percent negative. This result shows that 12 out of 150 transgenders are HBV positive while 138 out of 150 transgenders are HBV negative.

4.2 Prevalence of Hepatitis C in Transgender population

To find out the prevalence of hepatitis C virus in transgender population total 150 transgenders took part in screening camp. 21 transgenders out of 150 got positive HCV result while 129 transgenders are negative to hepatitis C virus. The overall prevalence for HCV in transgender population is 14%.

Results are summarized in Table 4.4 and Figure 4.4. The prevalence of HCV in transgender population is much high as compared to general population of Rawalpindi. Transgender population are at risk of developing other STD's because of their social behavior.

According to our findings 14% of transgenders are infected with hepatitis C virus in Rawalpindi but the rate of HCV infection will high in transgenders living in rural areas where there is no proper screening and counseling facility.

The prevalence of hepatitis C virus in transgender population of Indonesia is 6.5% [33]. Majority of transgenders have HCV along with HIV infection.

Table 4.4: Prevalence of Hepatitis C in Transgender Population.

	Frequency	**Percent**	**Valid percent**
HCV Positive	21	14%	14%
HCV Negative	129	86%	86%
Total	150	100%	100%

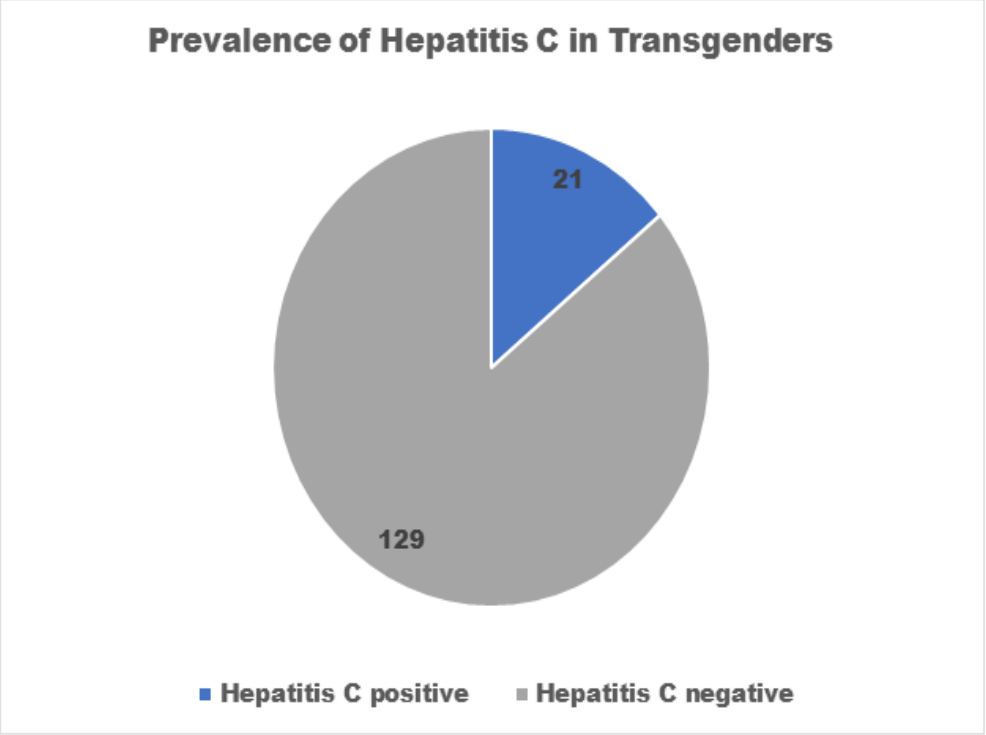

FIGURE 4.4: Prevalence of Hepatitis C in Transgender Population.

4.3 Prevalence of Hepatitis B Gender Wise

We have collected hepatitis B patient's data from hospital to find out the prevalence of HBV in male and female. The data of 100 patients was collected, 69 patients out of 100 belongs to male gender while 31 patients out of 100 belongs to female gender. These results indicate that the hepatitis B virus is more common in males than females. Table 4.5 and Figure 4.5 described our results.

The prevalence of HBV is common in males than in females according to our findings. Baig (2009), conducted an experiment and found that 79.5% males are positive to hepatitis B while 20.5% females are positive to hepatitis B infection [33]. The male to female ratio of hepatitis infection is very high. The high male to female ratio of HBV infection is due to occupational and social life of male.

Males are more exposed to viral infection when they go to barbers for facial and hair-cut. In Pakistan, males are also involved in injecting drug use.

TABLE 4.5: Prevalence of Hepatitis B Gender-wise.

	Male	**Female**	**Total**
HBV Positive	69	31	100

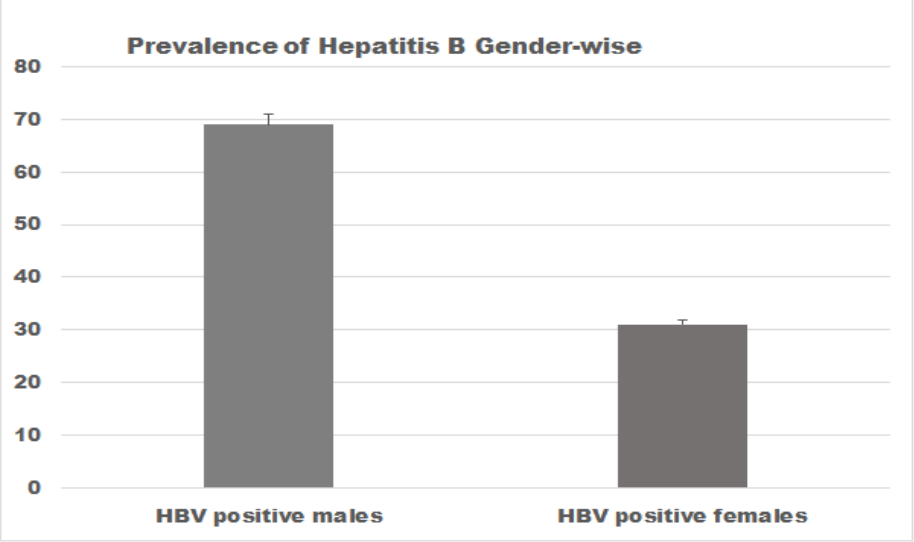

FIGURE 4.5: Prevalence of Hepatitis B Gender-wise.

4.4 Prevalence of Hepatitis C Gender Wise

To find out the prevalence of HCV in both male and females. The data of 100 HCV patients were collected from hospital to find out the prevalence. The result shows that 76 patients out of 100 are males and 24 patients of HCV out of 100 are females. These results show that hepatitis C virus is predominant in males as compared to females. Results are summarized in Table 4.6 and Figure 4.6.

Most commonly it is cleared that males are more infected with HCV than females. Males have more chances of developing viral infections because of their occupational and social behavior. Barbers and injecting drug use are the major risk

factors for HCV development in males. Females are in least number for HCV infection because due to high level of estrogen in females. The receptors of estrogen in the liver of females protect hepatocytes cells from cell death, oxidative stress and inflammatory injury which all leads to fibrosis. The estrogen receptors in the liver of females protect females from HCV infection [34].

TABLE 4.6: Prevalence of Hepatitis C Gender-wise.

	Male	**Female**	**Total**
HCV Positive	76	24	100

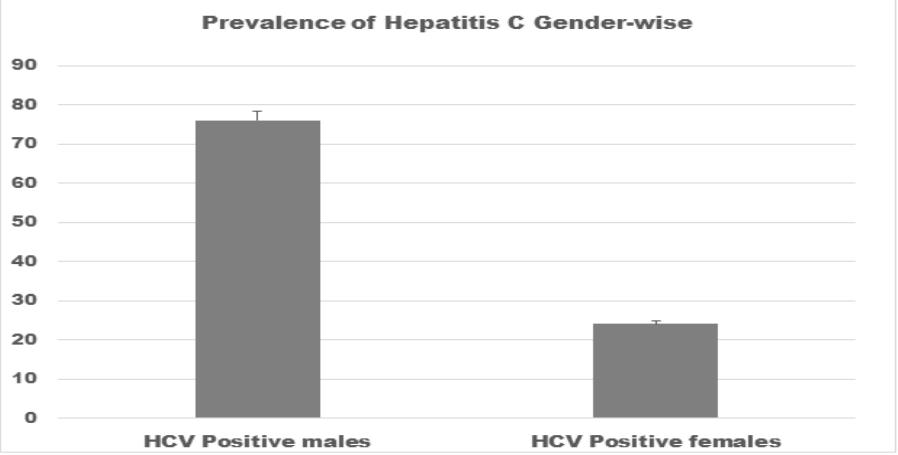

FIGURE 4.6: Prevalence of Hepatitis C Gender-wise.

4.5 Correlation of Hepatitis B and Age

To find out the relationship of Hepatitis B with age the following results produced. We collected data of 100 hepatitis B patients, 17 out of 100 belongs to age less than 40 while 83 patients out of 100 belongs to more than 40 age category. These findings indicate that hepatitis B is more common in people of more than 40 age. Results are discussed in Table 4.7 and Figure 4.7. The study conducted by Guclu et al (2015), the hepatitis C infection is high in people whose age more

than 40 as compared to people whose age less than 40. The risk of HCV and other viral infection increases with age. When age is more than 40 the immune system of a person is not much efficient to tackle the viral attack. There is a direct relationship between increasing age and susceptibility to infection. Weak immune system, degree of exposure to infection and anatomical and functional changes contribute to easily infect elderly person [35].

TABLE 4.7: Correlation of Hepatitis B and Age.

	Less than 40	**More than 40**	**Total**
HBV Patients	17	83	100

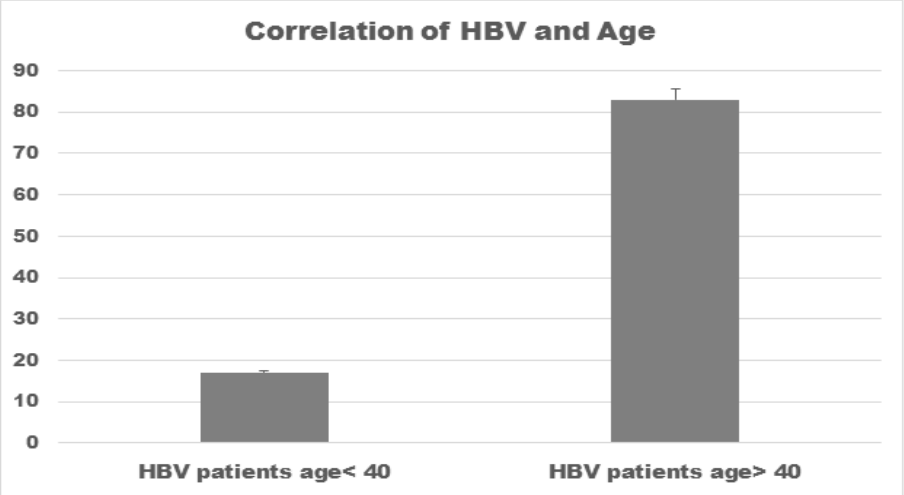

FIGURE 4.7: Correlation of HBV and Age.

4.6 Correlation of Hepatitis C and Age

To find out the correlation of Hepatitis C with age, we collected data of 100 HCV patients from hospital. 12 patients out of 100 belongs to less than 40 age category while 88 patients out of 100 belongs to more than 40 age category. These findings indicate that hepatitis C virus is more common is people of above the age of 40. Findings are discussed in Table 4.8 and Figure 4.8. The immune response specially

cell mediated immune response declines in efficiency with age. People with elderly age are more susceptible for developing infection due to weak immune system and anatomical and functional changes of body [36]. Different factors involved in morbidity and mortality in aged people are decrease host resistance, chronic underlying diseases, weak immune system and delays in diagnosis of diseases. The secondary health problems in elderly age people like diabetes, hypertension and cardiac disease are also able the body to immuno-compromised for different infections.

TABLE 4.8: Correlation of Hepatitis C and Age.

	Less than 40	**More than 40**	**Total**
HCV Patients	12	88	100

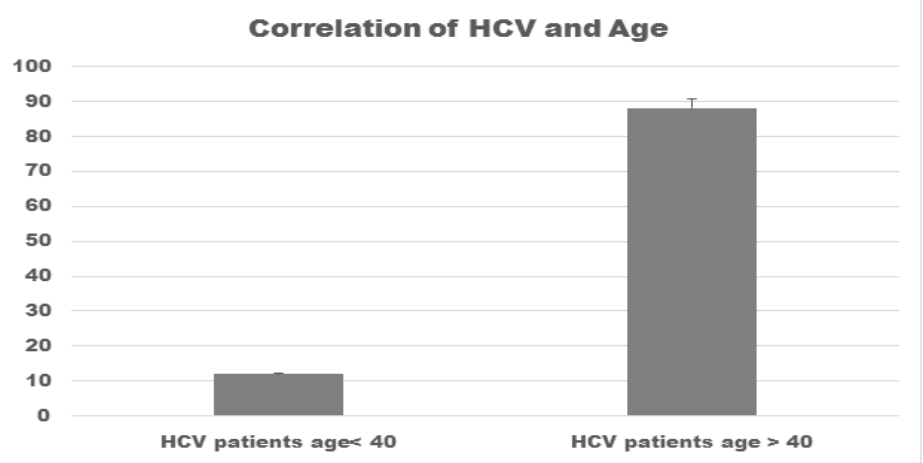

FIGURE 4.8: Correlation of HCV and Age.

4.7 Correlation of HBV and Blood Groups

To find out the prevalence of hepatitis B in different blood groups of the patients. We collected data of 100 HBV patients, most of the patients belong to blood group B and A. The most common blood group of HBV patient is B+ and A+, 22% and 18% respectively. The least common blood group of HBV patients are AB- and

O-. Our different findings are discussed in Table 4.9 and Figure 4.9. The study conducted by Batool et al state that people with blood group B positive are more infected with hepatitis B as compared to other blood groups [37]. In our study, patients with blood group B positive are also have a high rate of HBV infection than any other blood group. So, the blood group B positive may be susceptible for developing viral infections. A study conducted by Jing et al, people of Africa and some regions of Asia with blood group O are more susceptible to HBV infection. The risk for HBV infection is 12% increases in people with blood group O [38].

TABLE 4.9: Correlation of HBV With Different Blood Groups

Blood groups	A+	A-	B+	B-	AB+	AB-	O+	O-
No of Patients	18	08	22	18	10	04	16	04
Percentage	18%	08%	22%	18%	10%	04%	16%	04%

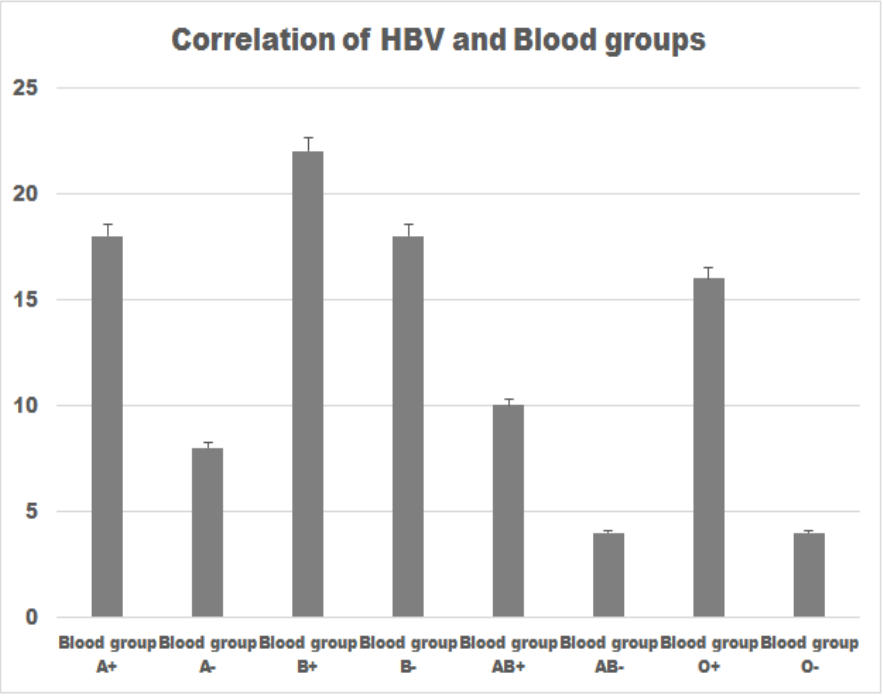

FIGURE 4.9: Correlation of HBV and Blood Groups.

4.8 Correlation of HCV with Blood Groups

In order to find out the prevalence of hepatitis C with respect to blood groups following findings obtained. We have collected data of 100 HCV patients, the more common blood group is B and A, 31% and 28% respectively. Blood group A and B are more common in the patients of hepatitis C virus. The least common blood groups are AB and O that are 24% and 17%. Obtained results are showed in Table 4.10 and Figure 4.10. The study conducted by Anwar et al, people with blood group A positive, B positive and O positive have high rate of HCV infection. The rate of infection in these blood groups are 8.15%, 7.57% and 8.96% respectively [39]. It is cleared that from both data the rate of HBV infection is high in Rh positive blood groups. In our data people with blood group A positive, B positive, AB positive and O positive have high rate of HCV infection. From these findings it is supposed that the Rh-positive factor contribute in viral infections.

TABLE 4.10: Correlation of HCV With Different Blood Groups

Blood groups	A+	A-	B+	B-	AB+	AB-	O+	O-
No of Patients	20	08	24	07	16	08	12	05
Percentage	20%	08%	24%	07%	16%	08%	12%	05%

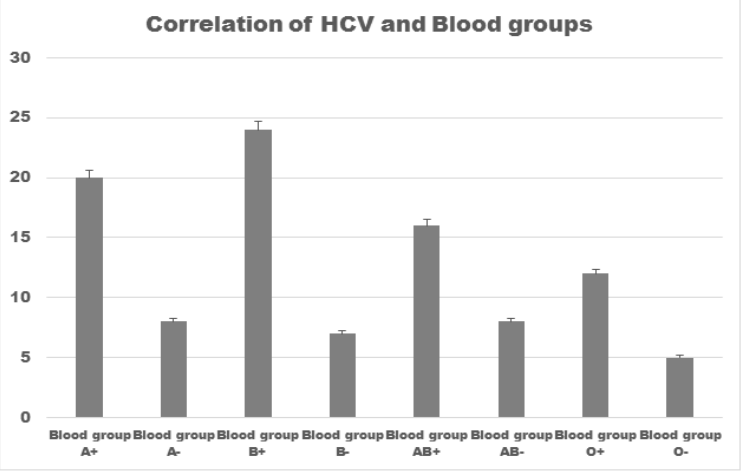

FIGURE 4.10: Correlation of HCV and Blood Groups.

4.9 Hemoglobin level in HBV patients

In order to find out the hemoglobin level in hepatitis B patients we collected data of 100 patients. We make 12 as a standard level of hemoglobin in adults. Our findings indicate that 28 patients have hemoglobin level less than 12 while 72 patients have hemoglobin level more than 12. This indicates that more patients have high hemoglobin level. Findings are discussed in Table 4.11 and Figure 4.11. The level of hemoglobin is high in most of the HBV patients. The body of patient produced more blood to help the immune system to fight with viral infection. High hemoglobin level indicates that body produces large amount of nucleated red blood cells that produces different molecules and triggers adoptive immune response. Red blood cells release some molecules that triggered innate immunity to fight against pathogens [40].

TABLE 4.11: Level of Hemoglobin in HBV Patients

Hemoglobin level	**Less than 12**	**More than 12**	**Total**
No of Patients	28	72	100

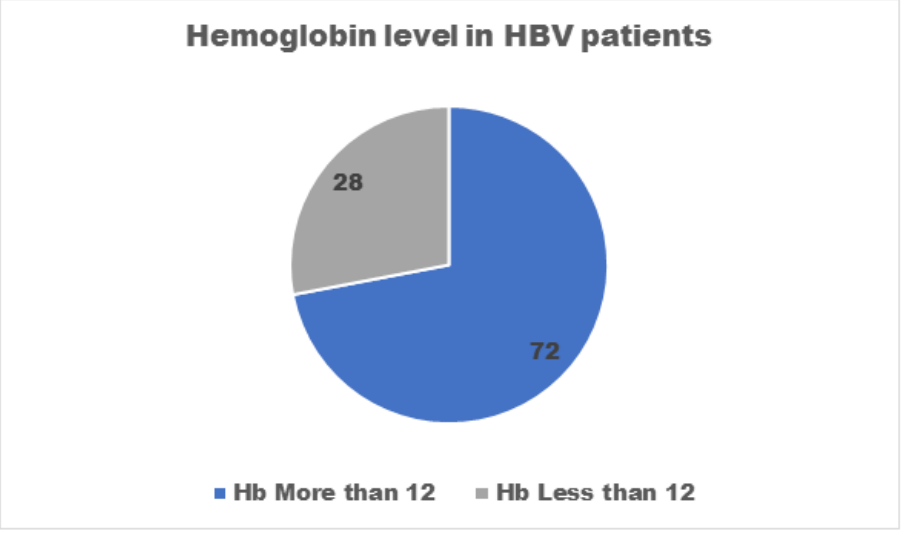

FIGURE 4.11: Hemoglobin Level in HBV Patients.

4.10 Hemoglobin level in HCV patients

In order to find out the hemoglobin level in hepatitis C patients, we collected data of 100 HCV patients from hospital. We make 12 as a standard level of hemoglobin in adults. According to our findings 22 patients have hemoglobin level less than 12 while 78 patients have hemoglobin level more than 12. Results are discussed in Table 4.12 and Figure 4.12. The HCV patients have high level of hemoglobin in their blood. The high level of hemoglobin indicates that body produces more blood. Body produces high amount of blood to tackle with the pathogen enter in the body. High level of hemoglobin means body produces large amount of red blood cells. These red blood cells release different molecules to activate the innate immunity of human body [40]. This innate immunity is the first line of defense and releases different components to tackle with pathogen enter in the body.

TABLE 4.12: Level of Hemoglobin in HCV Patients.

Hemoglobin level	**Less than 12**	**More than 12**	**Total**
No of Patients	22	78	100

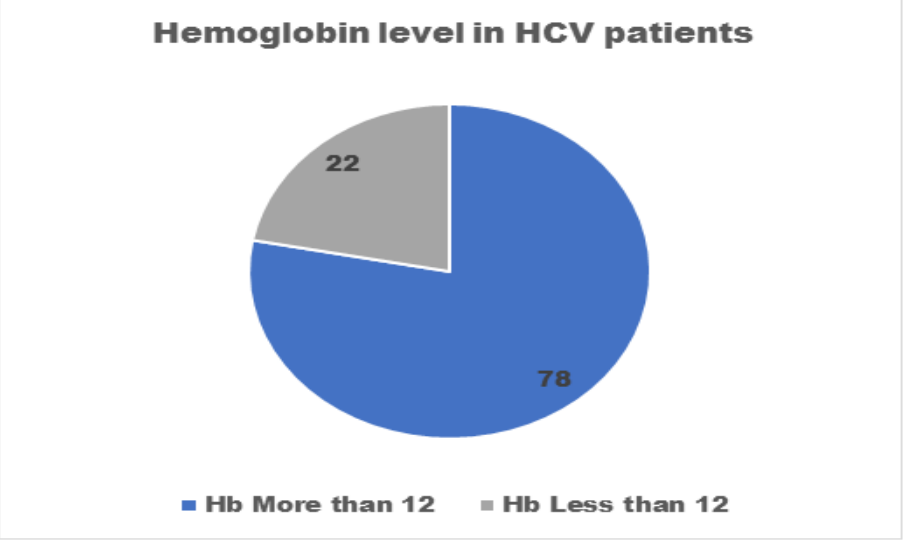

FIGURE 4.12: Level of Hemoglobin in HCV Patients.

4.11 Alanine Aminotransferase (ALT) in HBV Males

Alanine aminotransferase (ALT) is an enzyme (protein) that is particularly present in liver cells. ALT level is vary from person to person but normal range of ALT in males are 28-33 units per liter. In liver cells ALT converted protein into energy for liver cells. When the liver cells damage, the ALT releases from liver cells to blood stream. The level of ALT in blood stream indicates how much liver cells damage. Moura et al conducted study on hepatitis B patients to find out the level of ALT and aspartate aminotransferase (AST) in infected patients. The study revealed that most of the HBV patients had high ALT as well as AST level in blood stream [41]. In order to find out the ALT level in HBV males, we were collected data of 100 HBV patients. Out of 69 HBV male patients, 10 patients had ALT level less than 33 IU/L while 59 HBV males had ALT level more than 33 IU/L.

TABLE 4.13: ALT Level in Males Infected With HBV.

ALT in HBV Males	**Less than 33 IU/L**	**More than 33 IU/L**	**Total**
No of Patients	10	59	69

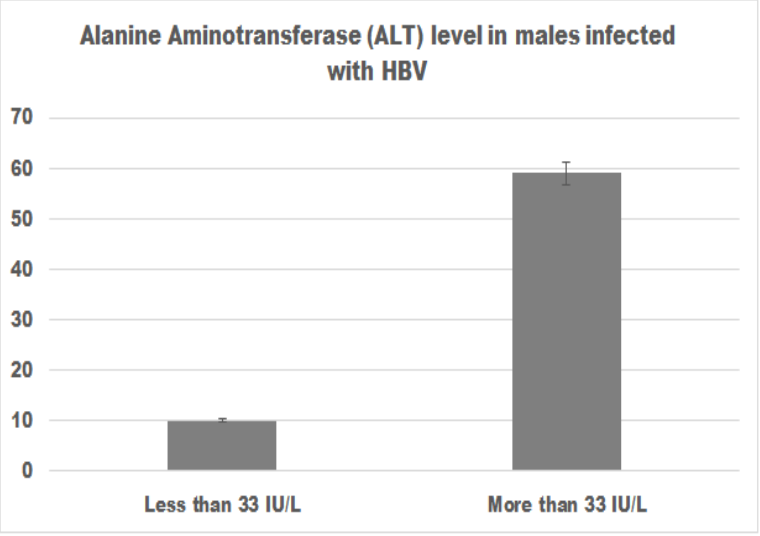

FIGURE 4.13: ALT Level in Males Infected With HBV.

4.12 Alanine Aminotransferase (ALT) in HBV Females

Alanine Aminotransferase (ALT) level in female patients infected with hepatitis B virus shows variation from person to person. ALT is the main enzyme of liver and its amount in blood indicates the health status of liver cells. The normal range of ALT in females are 19-26 units per liter in blood. Nguyen et al conducted meta-analysis on 683 patients and found that 48% patients had high level of ALT [42]. We were collected data of 100 HBV infected patients, 31 out of 100 were females. In female 4 patients had ALT level less than 26 IU/L while 27 patients had ALT level more than 26 IU/L. From these findings it is cleared that majority of females had ALT level more than from normal range. The results are discussed in Table 4.14 and Figure 4.14.

Table 4.14: ALT Level in Females Infected With HBV.

ALT in HBV Females	**Less than 26 IU/L**	**More than 26 IU/L**	**Total**
No of Patients	04	27	31

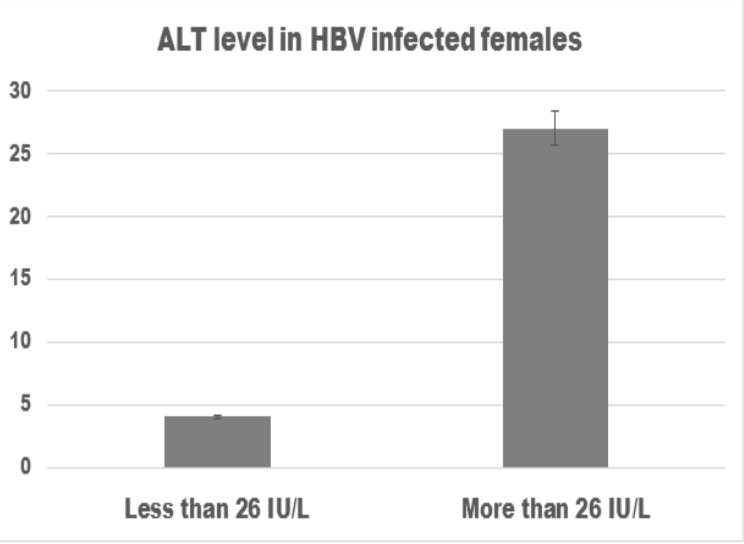

Figure 4.14: ALT Level in Females Infected With HBV.

4.13 Alanine Aminotransferase (ALT) in HCV Males

The level of Alanine aminotransferase level in hepatitis C patients shows wide range of variation. The hepatitis C patients usually shows high level of ALT in their blood. Hepatitis C virus damage the liver tissues and usually cause cirrhosis. Due to the damage of liver cells the high amount of ALT enzyme releases from hepatic cells to blood stream. According to study conducted by Akkaya et al, although the ALT level is high in most of the chronic HCV patients but 25% of chronic HCV patients have normal ALT level [43]. We collected data of 100 HCV patients, 76 out of 100 patients are males that were infected with HCV. According to our data 8 male patients had ALT level less than 33 IU/L while 68 male patients had ALT level more than 33 IU/L. Data shown in Table 4.15 and Figure 4.15.

TABLE 4.15: ALT Level in Males Infected With HCV.

ALT in HCV Males	Less than 33 IU/L	More than 33 IU/L	Total
No of Patients	08	68	76

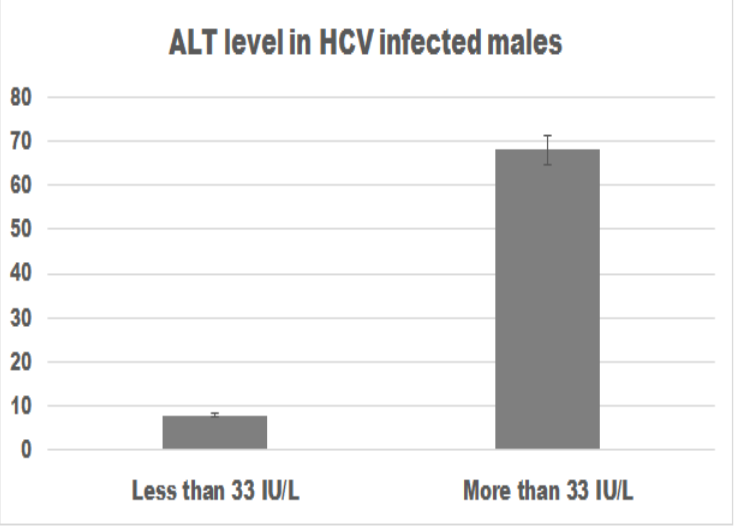

FIGURE 4.15: ALT Level in Males Infected With HCV.

4.14 Alanine Aminotransferase (ALT) in HCV Females

Alanine Aminotransferase is a protein-based enzyme that catalyzes protein and produced energy for hepatic cells. The normal range of ALT level in body indicates the health status of our liver. If the ALT level is high in blood indicates that our liver is damaged due to hepatitis virus, cirrhosis or other medical conditions. ALT usually present in hepatic cells of liver when the level of ALT is reported high in blood, this indicate that liver cell is being damaged and ALT releases into the blood stream. About 8% to 33% patients who are infected with chronic HCV have normal ALT level [44]. We were collected data of 100 HCV patients, 24 out of 100 were females. 3 patients out of 24 had ALT level less than 26 IU/L while 21 female patients had ALT level more than 26 IU/L. Results of our findings are represented in Table 4.16 and Figure 4.16.

TABLE 4.16: ALT Level in Females Infected With HCV.

ALT in HCV Females	**Less than 26 IU/L**	**More than 26 IU/L**	**Total**
No of Patients	03	21	24

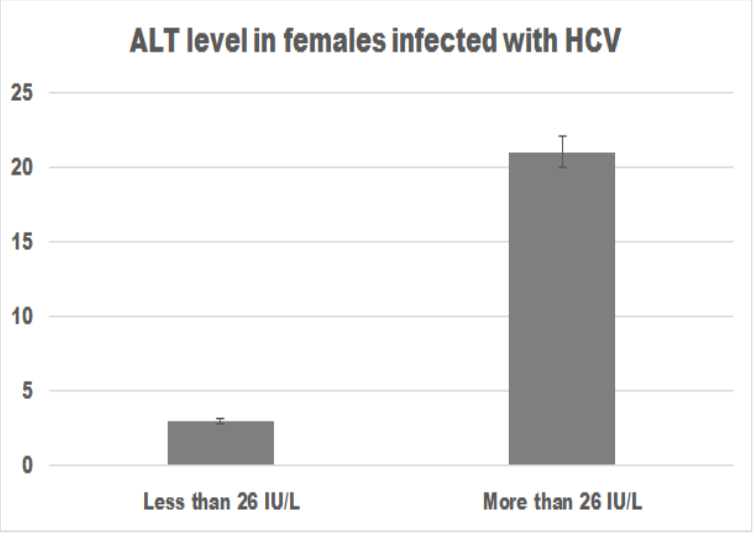

FIGURE 4.16: ALT Level in Females Infected With HCV.

4.15 Genotypes of Hepatitis B Virus in Rawalpindi

To find out the genotypes of hepatitis B virus present in patients of Rawalpindi city. We have collected data of 100 patients infected with hepatitis B virus. 60 patients have D genotype, 28 patients infected with C genotype while 12 patients infected with B genotype. Genotype A, E, F, G and H are not common in our country. Findings are listed in Table 4.13 and Figure 4.13. Different studies conducted in different regions of Pakistan indicates that most prevalent genotype of HBV infection in genotype D with prevalence rate of 63.7% [45]. According to Alam et al stated that most emerging genotype for HBV is genotype C with the prevalence rate of 27.66%. This is bad news as genotype C is more common in cirrhotic patients and causes severe liver damage. As the genotype D causes severe liver disease and less responsive to interferon-based therapy [46].

TABLE 4.17: Different Genotypes of HBV in Rawalpindi

Genotypes of HBV	A	B	C	D	E	F	G	H	**Total**
No of Patients	0	12	28	60	0	0	0	0	100

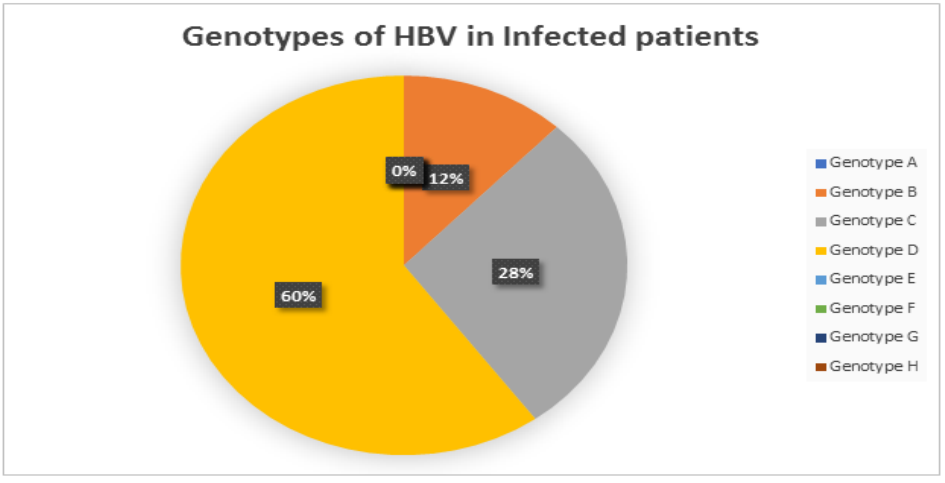

FIGURE 4.17: Genotypes of HBV in Infected Patients.

4.16 Genotypes of Hepatitis C Virus in Rawalpindi

To find out the most common genotypes of hepatitis C virus in Rawalpindi, We have collected data of 100 hepatitis C patients. According to data, 10 patients are infected with genotype 1, 4 patients are infected with genotype 2 and 86 patients are infected with genotype 3. Genotype 4, 5, and 6 are not common in our country. The most common genotype for hepatitis C virus infection is genotype 2. According to Khan et al, the most prevalent genotype for HCV in Swat region of Pakistan is genotype 3. The genotype 3a and 3b are the most prevalent genotype in Pakistani population. The data collected by Ataullah et al, the most prevalent genotype for HCV is genotype 3 with prevalence value of 78.96%, genotype 2 is 3.8% and genotype 1 is 7.04% respectively. High prevalence rate of HCV genotype 3 is a good hope for cure as well as control of HCV infection. Genotype 3 require shorter time period for treatment as compared to genotype 1 and 2. The cost of treatment is also reduced when treatment duration is shorter [47]. Our results also justify HCV genotype 3 is most prevalent in our population.

TABLE 4.18: Different Genotypes of HCV in Rawalpindi

Genotypes of HCV	1	2	3	4	5	6	**Total**
No of Patients	10	04	86	0	0	0	100

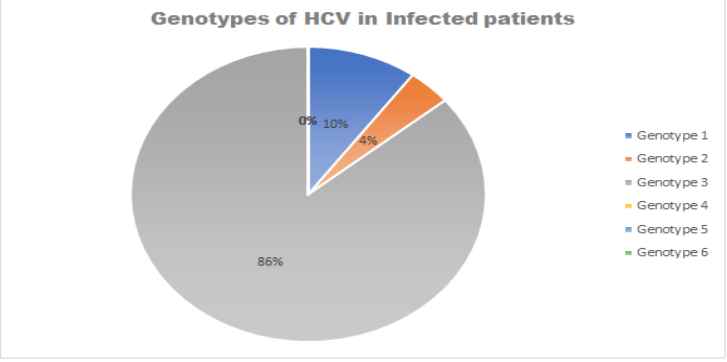

FIGURE 4.18: Genotypes of HCV in Infected Patients.

4.17 Association of HBV Genotypes with Age

To find out the correlation of HBV genotypes with the age of HBV patients we take data of 100 patients. We categorize the patients into two categories on the basis of their age one who have age less than 40 while the others have age more than 40. The data is summarized in Table 4.15 and Figure 4.19.

Total 17 HBV patients have age less than 40, 8 out of 17 are infected with D-genotype, 7 out of 17 are infected with C-genotype while only 2 patients out of 17 are infected with B-genotype. We apply Chi-square test on this data to find out that our data is significant or non-significant.

The chi-square value is 65.5641 and P-value is 0.000. As the P-value is less than from the standard 0.05 value, this means our result is significant.

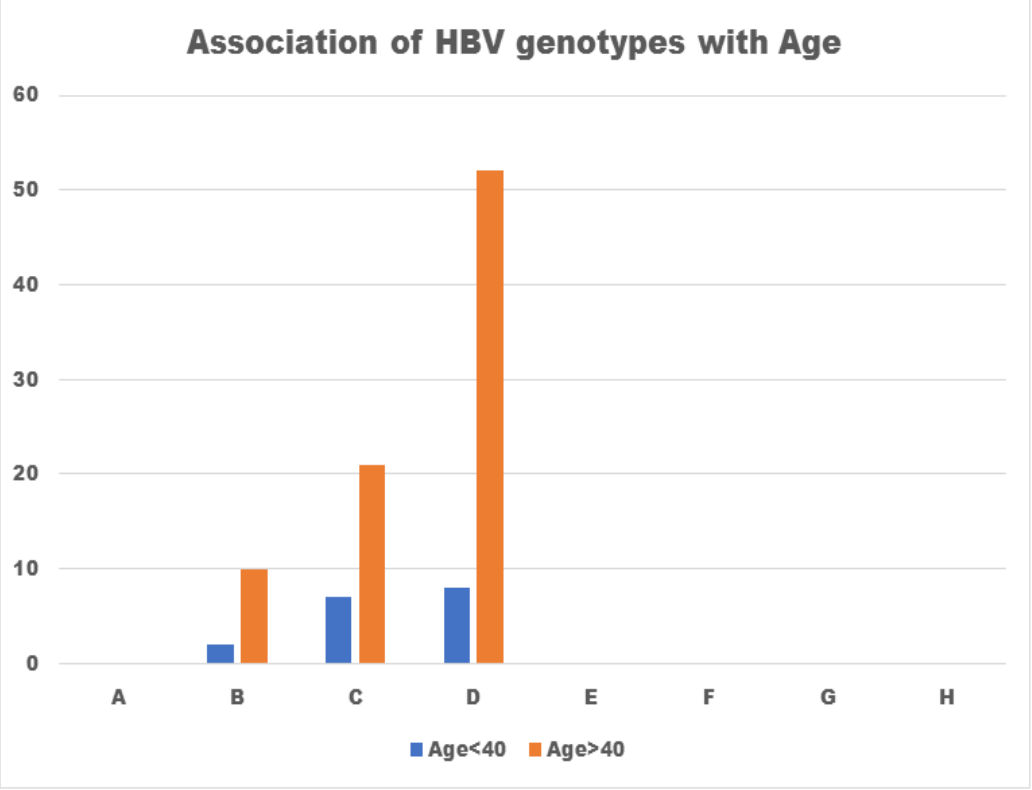

FIGURE 4.19: Association of HBV Genotype With Age.

Table 4.19: Association of HBV Genotypes With Age

	HBV Genotypes								Total	Chi-square	P-value
	A	B	C	D	E	F	G	H			
Age<40	0	02	07	08	0	0	0	0	17	65.5641	0.000
Percentage	0%	11.7%	41.1%	47%	0%	0%	0%	0%			
Age>40	0	10	21	52	0	0	0	0	83		
Percentage	0%	12%	25.3%	62.6%	0%	0%	0%	0%			

4.18 Association of HCV Genotypes with Age

To find out the correlation of HCV genotypes with age of HCV patients we collected data of 100 patients. We categorized age into two categories, one is less than 40 age while the other is more than 40 age. The overall data is discussed in Table 4.16 and Figure 4.20. Total 12 patients have age less than 40, 2 patients out of 12 are infected with Genotype 1, 9 patients out of 12 are infected with Genotype 3 and only one patient is infected with Genotype 2.

No patient belongs to Genotype 4 and 5. Total 88 patients have age more than 40, 8 patients are infected with Genotype 1, 77 patients are infected with Genotype 3 and only 3 patients are infected with Genotype 2. There is no patient that infected with genotype 4 and 5. We apply Chi-square test on our data to find the correlation of HCV genotypes with age of patients. The P-value is 0.487 that indicates our data is not significant as it is above the standard value of 0.05.

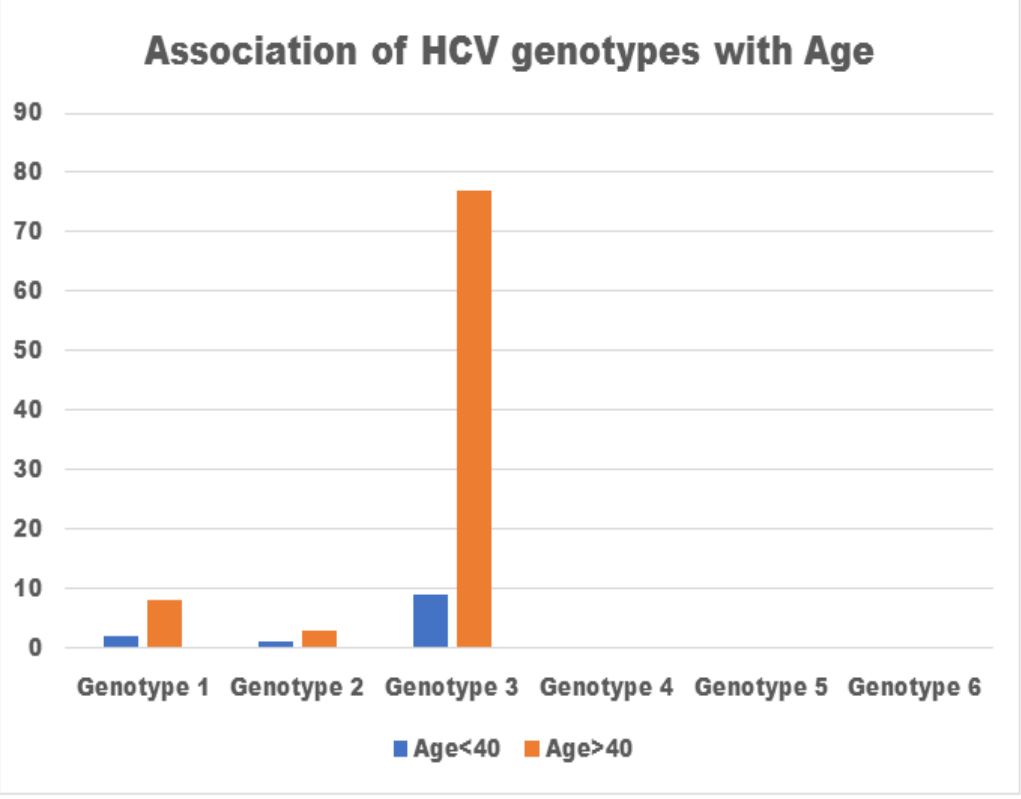

FIGURE 4.20: Association of HCV Genotype With Age.

Table 4.20: Association of HCV Genotype With Age

	HCV Genotypes						Total	Chi-square	P-value
	1	2	3	4	5	6			
Age<40	02	01	09	0	0	0	12	1.4381	0.487
Percentage	16.6%	8.3%	75%	0%	0%	0%			
Age>40	08	03	77	0	0	0	88		
Percentage	09%	3.4%	87.5%	0%	0%	0%			

4.19 Association of HBV Genotypes with Gender

To find out the correlation of HBV with gender, we have collected data of 100 HBV patients. 69 patients out of 100 are males while 31 patients are females. In males, 47 patients are infected with genotype D, 18 patients are infected with genotype C and only 4 patients are infected with genotype B. There is no patient that are infected with genotype A, E, F, G and H.

In females, 13 patients are infected with genotype B, 10 patients are infected with genotype C and only 8 female patients are infected with genotype B. We performed Chi-square test on our data to find out the correlation of HBV genotypes with gender as presented in Table 4.17. The P-value is 0.007 that is lower than the standard value 0.05 that indicate our data is significant. Results are shown in Table 4.17 and Figure 4.21.

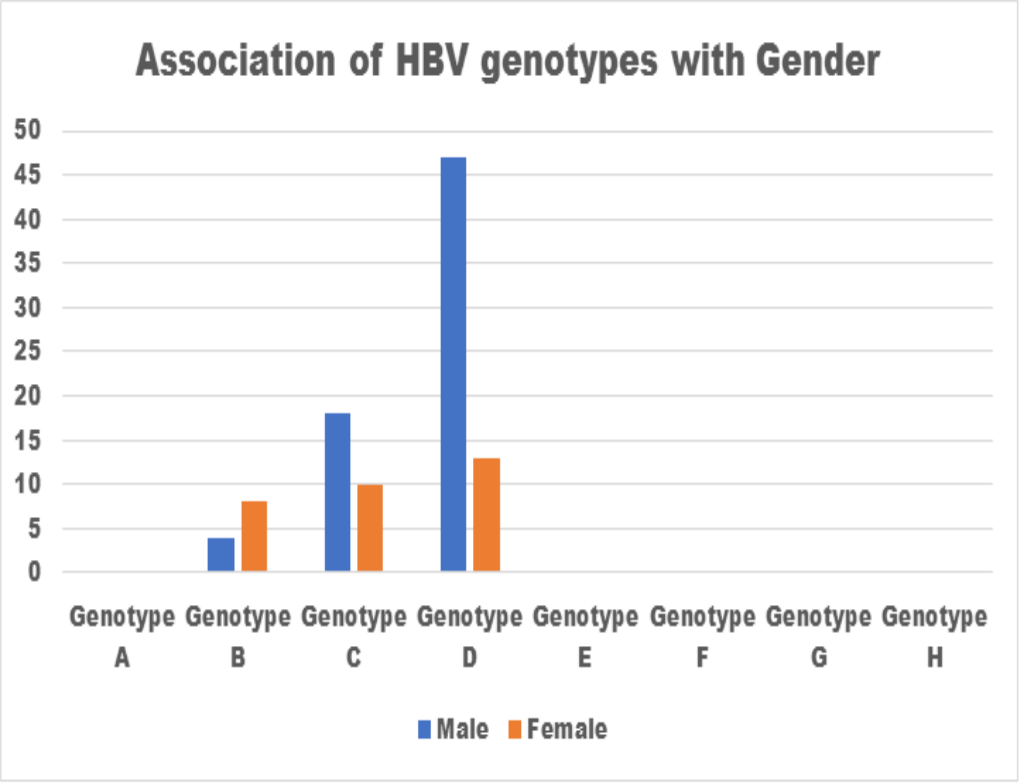

FIGURE 4.21: Association of HBV Genotype With Gender.

Table 4.21: Association of HBV Genotypes With Gender

	HBV Genotypes								Total	Chi-square	P-value
	A	B	C	D	E	F	G	H			
Male	0	04	18	47	0	0	0	0	69	9.8711	0.007
Female	0	08	10	13	0	0	0	0	31		

4.20 Association of HCV Genotypes with Gender

In order to find out the correlation of HCV genotypes with the age of patients, we have collected data of 100 HCV patients. 76 patients out of 100 are males while 24 patients are females. In 76 males, 4 patients are infected with genotype 1, 70 patients are infected with genotype 3 and only 2 male patients are infected with genotype 2. In females, 6 patients are infected with genotype 1, 16 patients are infected with genotype 3 and only 2 patients are infected with genotype 2.

In our data no male or female are infected with genotype 4 and 5. We apply Chi-square test on our data to find the correlation of HCV genotypes with gender as data presented in Table 4.18. The P-value is 0.006 which is less than the standard value of 0.05 that indicated our data is significant. Results are discussed in Table 4.18 and Figure 4.22.

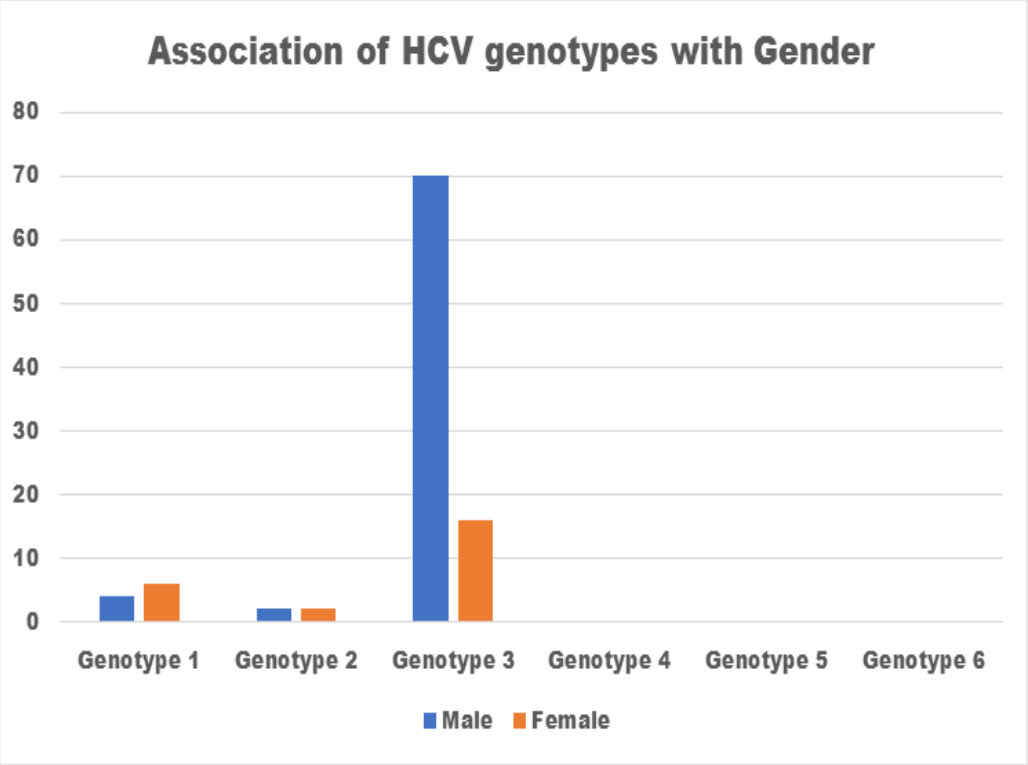

FIGURE 4.22: Association of HCV Genotype With Gender.

TABLE 4.22: Association of HCV Genotypes With Gender

	HCV Genotypes					Total	Chi-square	P-value
	1	2	3	4	5			
Male	04	02	70	0	0	76	9.9602	0.006
Female	06	02	16	0	0	24		

4.21 Discussions

In this study, we screened out 150 people of general population to find out the prevalence of hepatitis B and C in Rawalpindi city. The screening along with awareness carried out in different regions of Rawalpindi city.

Total 150 people both males and females took part in screening camp 3 people out of 150 shows positive result for hepatitis B while 147 people are negative to hepatitis B virus.

The overall percentage for hepatitis B positive in general population is 2% while 98% are negative to HBV. To find out the prevalence of hepatitis C virus in general population, we conducted screening of 150 people both males and females.

According to our findings, 16 people showed hepatitis C positive result while 134 people are negative to hepatitis C virus. The overall percentage for hepatitis C positive people are 10.6% while 89.3% are negative to hepatitis C virus.

From these results it is cleared that hepatitis C is most common in people than hepatitis B virus. Transgenders belong to discriminative community in Pakistan that faces social and legal discrimination. They are usually belongs to lower middle class status and many of them beg on streets or perform sex work to survive.

In order to find out the prevalence of hepatitis B and C in transgender population of Rawalpindi city. We conducted screening of 150 transgenders in different areas of Rawalpindi. 12 out of 150 transgenders are positive to hepatitis B virus while 138 transgenders are negative to hepatitis B virus. The percentage for HBV positive transgenders is 8% while the percentage for HBV negative transgender is 92%. The prevalence of hepatitis C virus in transgender is very high because of their social and behavioral practices.

Wo screened out 150 transgenders, 21 of them are positive to hepatitis C virus while 129 transgenders are negative to hepatitis C virus. The percentage for HCV positive transgender is 14% while 86% transgenders are negative to HCV. From

these findings it is cleared that hepatitis C virus is more common than hepatitis B virus in transgenders.

The percentage for HCV positive transgender is 14% while 86% transgenders are negative to HCV. From these findings it is cleared that hepatitis C virus is more common than hepatitis B virus in transgenders. For further investigations we collected data of hepatitis B and hepatitis C patients from hospital to analyze further parameters. We have collected data of 100 hepatitis B patients including males and females.

69 males and 31 females are infected with hepatitis B virus. So, the percentage of HBV is high in males as compared to females. According to gender-wise study HBV is more common in males than females. The data of HCV patients has been collected from hospital to find out the prevalence of HCV in both genders. Data of 100 HCV patients collected 76 patients are male while 24 patients are females. It is cleared that HCV is more common in males than in females.

To find out the relationship of age with hepatitis B viral infection. We have collected data of 100 patients, 17 patients out of 100 have age less than 40 while 83 patients have age more than 40.

From this data it is cleared that hepatitis B is more common in those persons whose age is more than 40. In the same way, we find out the prevalence of hepatitis C with respect to age. We collected data of 100 HCV positive patients, 12 out of 100 have age less than 40 while 88 patients out of 100 have age more than 40. So, hepatitis C virus is more common in those people whose age is more than 40.

In order to find out the prevalence of hepatitis B and C in different blood groups of people. We have collected data of 100 HBV patients that belongs to different blood groups.

18 patients have A+ blood group, 8 patients have A- blood group, 22 patients have B+ blood group. 18 patients have B- blood group, 10 patients have AB+ blood group, 4 patients have AB- blood group, 16 patients have O+ blood group

while 4 patients have O- blood group. So most of the patients belongs to B+, A+ and A- blood group while few patients belongs to O-, AB- and A- blood group.

In the same way to find out the prevalence of hepatitis C with respect to blood groups of HCV patients, we have collected data of 100 HCV patients 20 patients have A+ blood group, 8 patients have A- blood group, 24 patients have B+ blood group, 7 patients have B- blood group, 16 patients have AB+ blood group, 8 patients have AB-, 12 patients have O+ and only 5 patients have O- blood group. From these findings it is clear that B+ and A+ are most common blood groups in HCV patients while few patients belongs to A-, AB- and O- blood group. As we know that hepatitis B and C virus infect the liver which is the major organ of our body. To find out the hemoglobin level in hepatitis B patients, we collected data of 100 hepatitis B patients.

We make a standard 12g/dL of hemoglobin level. From our data 28 patients have hemoglobin level less than 12g/dL while 72 patients have hemoglobin level more than 12g/dL. In this way it is cleared that 72% patients have hemoglobin level more than 12g/dL.

Most of the patients of HBV have high hemoglobin level. So to find out the hemoglobin level in hepatitis C patients we have collected data of 100 patients 78 patients have hemoglobin level more than 12g/dL while 22 patients have low hemoglobin level than 12g/dL. The hemoglobin level in most of the hepatitis B and C patients have more than 12g/dL.

There are eight genotypes of hepatitis B virus from A to H. The genotypes of HBV are distributed is all over the world. Genotype C is most common genotype worldwide. Genotype B and C are most common in Asia [19]. To find out the most common genotype in Rawalpindi, we have collected data of 100 HBV patients, 60 patients out of 100 have genotype B, 28 patients have genotype C and 12 patients have genotype D. There is no patient that have genotype A, E, F, G and H. So, the most common genotype of HBV is genotype B in our population. Globally hepatitis C virus strain are classified into six recognized genotypes (1-6) on the basis of phylogenetic and sequence analysis of whole viral genome [43].

In the same way we also try to find out the most common genotype of hepatitis C in Rawalpindi. We have collected data of 100 HCV patients to predict most common HCV genotype in our population. According to our findings 86 patients are infected with genotype 3, 10 patients are infected with genotype 1 while only 4 patients are infected with genotype 2. There is no patient infected with genotype 4, 5 and 6. Now this is clear that the most common genotype of HCV in our population is genotype 2.

Chapter 5

Conclusions and Recommendations

Conclusion to this research was that in general population of Rawalpindi the prevalence of hepatitis C (10.6%) is more than hepatitis B ((2%). In transgenders population the prevalence of hepatitis C (14%) is also more than hepatitis B (8%). According to our findings, males are more infected with hepatitis B and C as compared to females. Most of the HBV and HCV infected patients have age more than 40 years. Most of the HBV and HCV infected patients have blood group A and B. Most of the HBV and HCV patients have hemoglobin level more than 12g/dL. Most of the HBV and HCV patients have high ALT level. In our population most common genotype of hepatitis b virus is Genotype B while most common genotype of hepatitis C virus is Genotype 3.

5.1 Recommendations

As the infection of HBV and HCV infect million of people in Pakistan. It is recommended to speed up the screening procedure of HBV and HCV in Pakistani population to overcome the rate of infection. Unfortunately, the screening of HBV and HCV in Pakistan is very costly and government do not play any role to

being a part of international campaign “Missing millions”. Seminars and trainings should be conducted to educate the people about viral hepatitis and their mode of transmission.

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Appendix A

DATA ACQUISITION FORM FOR RESEARCH PROJECT

Project Title: Prevalence of Hepatitis B and C in General population and Transgender population of Rawalpindi.

BIODATA: (This information provided by patient will be confidential)

Patient Name: _____ Age: _____ Gender: _____

Contact no: _____ City: _____ Province: _____

CLINICAL INFORMATION

1. When was the disease first diagnosed?

(a) Below the age of 20

(b) Above the age of 20

(c) Don't know

2. Do you have any other infection instead of HBV and HCV?

(a) Yes

(b) No

(c) Don't Know

3. Do you think that HBV and HCV transmit by using unsterilized syringes or medical equipment?

(a) Yes

(b) No

(c) Don't know

4. Blood group of patient ————

5. Patients hemoglobin level ————

6. Alanine transaminase (ALT) level of patient ————

7. Genotype of HBV————

8. Genotype of HCV————