Identification of Drug Resistant Mutations among HIV-1 infected Individuals in Pakistani population

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A thesis submitted in partial fulfilment of the requirement for the degree of

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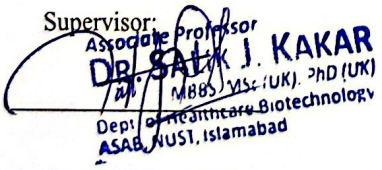

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I hereby declare that except where specific reference is made to the work of others, the content of this dissertation is original and have not been submitted in whole or in part for consideration for any other degree or qualification in this or any other University. This dissertation is the result of my own work and includes the outcome of the work done.

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This thesis is dedicated to all those who have hope and

have ever dared to dream big

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Muhammad Moaaz Anwar

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

HIV Human Immunodeficiency Virus

AIDS Acquired Immune Deficiency Syndrome

ART Anti-Retroviral Therapy

ARVs Anti-Retroviral drugs

PrEP Pre-Exposure Prophylaxis

PEP Post-exposure prophylaxis

CRFs Circulating Recombinant Forms

HIV-1 Human Immunodeficiency Virus type-1

HIV-2 Human Immunodeficiency Virus type-2

DRMs Drug Resistance Mutations

PLHIV People Living with Human Immunodeficiency Virus

MSW Male Sex Workers

FSW Female Sex Workers

TGSW Transgender Sex Workers

MSM Men who sex with Men

PMTCT Prevention of Mother-to-Child Transmission

IDUs Injectable Drug Users

NGOs Non-Government Organizations

KS Kaposi's sarcoma

LAV Lymphadenopathy-Associated Virus

HTLV-III Human T Lymphotropic Virus-III

CD4+ Cluster of Differentiation 4

RNA Ribonucleic Acid

5′ UTR 5′ Untranslated region

SU Surface Unit

TM Transmembrane Unit

3′ UTR 3′ Untranslated region

mRNA Messenger Ribonucleic Acid

gRNA Genomic Ribonucleic Acid

LTRs Long Terminal Repeats

MA Matrix

CA Capsid

NC Nucleocapsid

PR Protease

RT Reverse Transcriptase

IN Integrase

TU Transmembrane Unit

DNA Deoxyribonucleic Acid

CCR-5 C-C Chemokine Receptor 5

PIC Pre Integration Complex

ESCRT Endosomal Sorting Complex Required for Transport

INSTI Integrase Strand Transfer Inhibitor

NNRTI Non-Nucleoside Reverse Transcriptase Inhibitor

NATs Nucleic acid tests

NRTI nucleoside reverse transcriptase inhibitor

PI Protease Inhibitors

WHO World Health Organization

cDNA complementary DNA

dNTPs Deoxynucleoside triphosphates

PCR Polymerase Chain Reaction

NCBI National Center for Biotechnology Information

MEGA Molecular Evolutionary Genetics Analysis

HIVDB Human immunodeficiency Virus Data Base

RPV Rilpivirine

NVP Nevirapine

3TC Lamivudine

ETR Etravirine

FTC Emtricitabine

EFV Efavirenz

ABC Abacavir

ABSTRACT

According to UNAIDS 38.4 million individuals infected with HIV all over the globe in 2021 and worldwide AIDS epidemic is still growing. As a result, Pakistan, which has one of Asia's highest HIV prevalence estimates, is seeing a fast shift in HIV transmission (from "low prevalence, high risk" to "concentrated" HIV pandemic status). Only 17,149 of the country's 165 000 HIV-infected individuals were receiving antiretroviral therapy (ARVs) as of 2019. Antiretroviral drugs (ARVs) have been smuggled throughout the nation due to inadequate governance and insufficient management, making them available to nearly anybody without a prescription. HIV Drug Resistance and the formation of Drug Resistant Viral Isolates have emerged from self-medication because of related stereotyping, drug overdose, and poor medication management. Transmitted Drug Resistance related mutations, also known as primary mutations, is a serious kind of drug resistance. Inability to screen populations for these basic alterations nearly always leads to the development of additional drug-resistant variants. Because no trustworthy statistics on this topic has yet been obtained or published, the primary goal of this project is to test HIV-infected Pakistani individuals for antiretroviral treatment resistance mutations.

INTRODUCTION

HIV & AIDS

Human immunodeficiency virus (HIV) is a virus that attacks immune system cells, making a person more susceptible to a variety of illnesses. It is transmitted through an exchange of injecting apparatus or through interacting with certain body fluids of an HIV-positive person, most commonly during unprotected sexual activity (sexual activity without the use of a condom or HIV medication to prevent or treat HIV). AIDS may result if HIV is not adequately treated (Stoff, Khalsa, Monjan, & Portegies, 2004).

Antiretroviral therapy, or ART, is still available as an effective HIV treatment option. If taken as instructed, treatment for HIV can substantially reduce the viral load, another term for the amount of HIV in the blood. This is known as viral suppression. When a person's viral burden is so minimal that it cannot be detected by a conventional laboratory, they are said to have an undetectable viral load. People with HIV can live long, healthy lives and will not transmit the virus to their HIV-negative companions if they take their HIV medications as prescribed while retaining an undetectable viral load (Volberding & Deeks, 2010).

In addition, there are efficient ways of preventing the spread of HIV via sex or drug use, such as pre-exposure prophylaxis (PrEP), a drug that people at risk for contracting HIV take to avoid contracting HIV via sex or injecting drugs, and post-exposure prophylaxis (PEP), a drug that HIV-positive individuals must take within 72 hours of an increased risk to prevent the virus from setting up a foothold.

Classification of HIV

The genus Lentivirus, subfamily Orthoretrovirinae of the Retroviridae family, is where the human immunodeficiency virus (HIV) is classified. HIV is divided into types 1 and 2 according to genetic attributes and variations in the viral proteins (HIV-1, HIV-2).

HIV-1 is divided into three classes: group M (main), group O (outlier), and group N. (new). The far more varied group, Group M, is made up of nine subtypes (A-D, F-H, J, and K) and many circulating recombinant forms (CRFs) (Bbosa, Kaleebu, & Ssemwanga, 2019). The prevalence of the subtypes geographically and demographically is varied, including one or more variants dominate infection in certain geographical regions; for instance, subtype C predominates in Southern and Eastern Africa, as well as China. The incidence of non-B subtypes and infection is expected to rise in the industrialized western world, along with the United States, because of increased rates of mobility and interaction with people from non-B endemic areas. Regarding this tendency, most research-based efforts have been focused on the geographically prevalent subtype B in North America and Europe. According to recent studies, subtypes C and A together account for more than 70% of all new infections, whereas HIV-1 subtype B only accounts for 12% of the estimated 40 million HIV-infected people globally (Kilmarx, 2009).

DRM's & Pakistan

According to estimates, 183,705 persons in Pakistan are HIV positive (PLHIV). People who inject drugs, male, female, and transgender sex workers (MSW, FSW, & TGSW), men who have sex with men (MSM), and transgenders are among the primary segments in a

society where the outbreak of HIV revolves in the United States. The HIV pandemic in Pakistan is moving into the sexual networks from where it is quietly spreading to the public via bridge populations. This is in line with the Asian Epidemic Modelling pattern. The epidemic has almost reached a plateau among drug injectors.

Antiretroviral treatment (ART) advancements have led to notable successes, among them the significant reductions in morbidity and death seen in HIV-infected individuals. The virus's main survival strategy, which derives from its enormous ability to produce variation, is antiretroviral (ARV) medication or drug resistance (Volberding & Deeks, 2010) . An evaluation of 117 sources has provided information on the general frequency of ARV resistance in the developing world, with an emphasis on treatment-naive individuals, the effects of prevention of mother-to-child transmission (PMTCT) drug regimens on resistance, and the connection between medication adherence and resistance. Global treatment-naive populations' patterns of ARV resistance seem to match regional patterns in the use of ARV drugs. The frequency of resistance (to any medicine) among people who have never had treatment was reported to be 5.5% in Africa, 7.4% in East Asia, 5.7% in Southeast Asia, and 6.4% in Latin America, lower than the rates in North America (11.4%) and Europe (10.6%) (Gregson et al., 2016) .

There were insufficient resistance data for HIV clades other than A, B, C, and D to allow for trustworthy findings. The prevalence of male sex workers and injectable drug users (IDUs) is soaring in Pakistan (MSWs). Although ART medications have been trafficked into Pakistan since the early 1990s and are accessible there without a prescription, ARV medications have been provided free of charge in Pakistan since 2004 through the National AIDS Control Program with assistance from the Global Fund to fight AIDS, Tuberculosis, and Malaria. A

few nongovernmental organizations (NGOs) have been sporadically giving HIV-positive people ARV medications. Based on their availability, some pharmacies might provide ARV medications to patients as accessible pharmaceuticals. In the nation as of February 2010, there were 1381 patients taking ARV medications, and 3176 people had signed up to do so (National AIDS Control Program, Ministry of Health). There are, however, limited research on the prevalence of HIV subtypes in Pakistan and no comprehensive data on ARV medication resistance in the nation. To direct testing and therapy decisions, local knowledge of resistance is required. Furthermore, differences in disease progression rates, responses to antiretroviral treatment (including the emergence of resistance), and vaccine development might all be affected by viral variety (Abidi et al., 2021).

1.1 Aims and Objectives

The study's goals are to:

- Collect and describe HIV Drug Resistance patterns and variables in the Pakistani population.
- To assemble complete data on drug resistance mutations that are circulating in Pakistan.

LITERATURE REVIEW

2.1 HIV Historical Background

The human immunodeficiency virus (HIV) was first identified in the early 1980s. In 1981, cases of a rare lung infection called Pneumocystis pneumonia (PCP) began to appear in large numbers among gay men in Los Angeles. At the same time, cases of a rare cancer called Kaposi's sarcoma (KS) began to appear among gay men in New York City. Both illnesses were rare before 1981, and their sudden appearance in large numbers of previously healthy gay men was a cause for concern (Weber, Wang, & Harrison, 2021).

In 1983, French virologist Luc Montagnier and his team at the Pasteur Institute in Paris discovered a new virus in the blood of a man with AIDS. They named the virus lymphadenopathy-associated virus (LAV). In 1984, American scientist Robert Gallo and his team at the National Cancer Institute in Maryland announced that they had discovered a virus called human T-lymphotropic virus-III (HTLV-III) that was causing AIDS. In 1986, it was determined that LAV and HTLV-III were the same virus, which was subsequently named human immunodeficiency virus (HIV) (Schipani-McLaughlin, Lambert, Lauckner, & Hansen, 2017).

Since the discovery of HIV, much has been learned about the virus and how it causes AIDS. Today, there are effective treatments that can slow the progression of HIV infection and prolong the lives of people living with AIDS. However, there is still no cure for HIV, and the epidemic continues to be a major global health concern.

2.2 Structural & genome organization of HIV

HIV, or human immunodeficiency virus, is a retrovirus that infects and attacks immune cells, specifically CD4+ T cells, which play a critical role in the adaptive immune response. The virus is spherical in shape and is approximately 120 nanometers in diameter. The viral envelope is composed of a lipid bilayer, which is studded with viral proteins, including the transmembrane protein gp41 and the surface glycoprotein gp120.

The genome of HIV-1, the virus that causes AIDS, is a single-stranded RNA molecule that is approximately 9.8 kilobases in length. It is a positive-sense RNA, meaning that it can act as a template for the synthesis of viral proteins and for the synthesis of a complementary negative-sense RNA strand (Schipani-McLaughlin et al., 2017).

a) The genome of HIV-1 is divided into three regions: the 5' untranslated region (5' UTR), the coding region, and the 3' untranslated region (3' UTR).
b) The 5' UTR contains the TAR element and the RRE element that are important for the regulation of viral gene expression.
c) The coding region is divided into three main regions:
d) The gag region codes for the core proteins of the virion, including the matrix, capsid, and nucleocapsid proteins.
e) The pol region codes for the viral reverse transcriptase, integrase, and protease.
f) The env region codes for the viral envelope proteins, including the surface (SU) and transmembrane (TM) proteins.
g) The 3' UTR contains the regulatory elements that control the production of viral mRNAs and the packaging of viral RNAs into virions.

In addition to the structural proteins and enzymes encoded by the HIV-1 genome, the virus also encodes several regulatory proteins, including the Tat and Rev proteins, that control the expression of viral genes and the replication of the virus (Schipani-McLaughlin et al., 2017).

Overall, the genetic information of HIV-1 is quite complex, but the knowledge of its genome organization has been important in the study and development of antiviral drugs, vaccines, and other therapies.

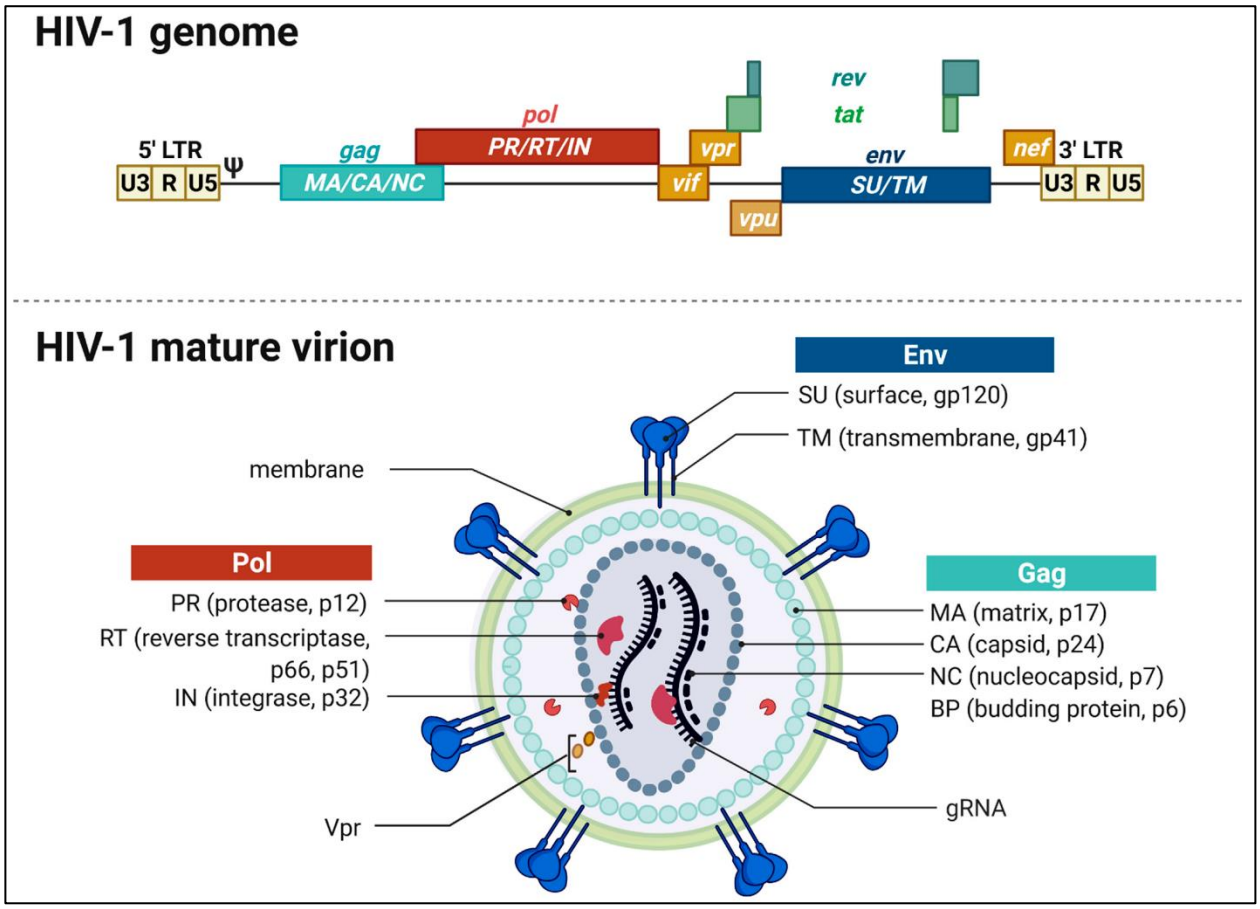

Figure 2.3 : *The construct of the HIV-1 genome and virion. (Top) A diagram of how the HIV-1 genetic makeup is put together, showing the open reading frames that code for the functional, regulatory, and accessory proteins. The dimeric, linear gRNA is about 9 kb long and is surrounded by 5'- and 3'-long terminal repeats (LTRs) that encompass the viral promoter and*

sequences needed for reverse transcription, integration, and gene expression. There are cis-acting regulatory elements in the U3, R, and U5 regions of the LTRs, which are followed by the packaging signal Psi (). Gag codes for the three structural proteins that make up the viral core: matrix (MA), capsid (CA), and nucleocapsid (NC). The enzymes protease (PR), reverse transcriptase (RT), and integrase are all made by viruses (IN). The two control genes, rev and tat, and the three helper genes, vif, vpr, and vpu, come after the Pol gene. Env codes for the surface unit (SU) gp120 and the transmembrane unit (TU) gp41, which are both parts of the viral envelope. The gene nef comes after the accessory gene env. (Bottom) The mature enveloped virion is in the shape of a sphere and is surrounded by a lipid bilayer membrane made from the host cell. This membrane has 7–35 trimers of envelope glycoproteins. Gag-made MA proteins are held in place by the inner layer of the membrane, which also holds Vpr and PR. The two copies of gRNA, RT, and IN are in the capsid, which is in the middle of the virion. The NC proteins keep the gRNA from moving around.(van Heuvel, Schatz, Rosengarten, & Stitz, 2022)

2.3 Types of HIV

There are two main types of the human immunodeficiency virus (HIV), which are known as HIV-1 and HIV-2.

HIV-1 is the more virulent and most common of the two types. It is responsible for most HIV infections globally. HIV-1 is divided into several subtypes, also known as clades, which are designated by letters of the alphabet (e.g., A, B, C, D, etc.). Some of the most common clades of HIV-1 include clade B, which is the most common in North America and Europe, and clade C, which is the most common in Africa and Asia (Eberle & Gürtler, 2012).

HIV-2 is less common than HIV-1 and is primarily found in West Africa. It is less virulent than HIV-1 and is associated with a slower progression to AIDS. HIV-2 is also divided into several subtypes, which are designated by numbers (e.g., HIV-2 group A, group B, etc.).

Both types of HIV are transmitted through the exchange of bodily fluids, such as blood, semen, vaginal fluids, and breast milk. They infect the same types of cells, CD4+ T-lymphocytes, and cause the same disease, AIDS, but their clinical manifestation, rate of progression and response to treatment are different.

HIV-1 subtype B is the most common in North America and Europe, it is the type of HIV used in most of the research and therefore, the most studied, hence more is known about its characteristics and response to therapy. While HIV-2 is less common, it is still present in some parts of the world and people infected with it require different treatments.

2.4 HIV subtypes

HIV-1 is divided into several subtypes, also known as clades, which are designated by letters of the alphabet (e.g., A, B, C, D, etc.). These subtypes are based on the genetic differences in the viral envelope gene (env).

The most common subtypes of HIV-1 are:

- Clade B: This is the most common subtype in North America and Europe and is responsible for most HIV infections in the developed world.
- Clade C: This is the most common subtype in Africa and is responsible for most HIV infections in the developing world.
- Clade A, D, F and G: These are fewer common subtypes that are primarily found in certain regions of the world.

- Clade O and N: These are rare subtypes that are primarily found in central Africa.

Each subtype of HIV-1 has its own unique genetic characteristics, which can affect the way the virus is transmitted and the course of the disease. For example, certain subtypes may be virulent or responsive to certain types of treatment. This information is important to understand while making recommendations for testing, prevention, and management of HIV-1 infection globally.

HIV-2 is also divided into several subtypes, which are designated by numbers (e.g., HIV-2 group A, group B, etc.). But these subtypes are much less diverse than those of HIV-1. These subtypes have been mostly described in West Africa and have unique characteristics that are not shared with HIV-1 subtypes, such as lower viral replication and different genetic markers (Drylewicz et al., 2008).

As the knowledge about different subtypes of HIV-1 and HIV-2 is important for the effective diagnosis, treatment, and management of patients with HIV-1 and HIV-2 infections, worldwide, it is an active area of research.

2.5 HIV & AIDS

HIV, or human immunodeficiency virus, is a virus that attacks the immune system and weakens the body's ability to fight off infections and certain cancers. The virus can be transmitted through blood, semen, vaginal fluids, and breast milk.

HIV, or human immunodeficiency virus, causes a major problem around the globe by attacking and weakening the immune system, making individuals infected with the virus much more susceptible to other infections and cancers. HIV primarily spreads through unprotected sexual contact, sharing of needles or other injection equipment, and from mother to child during

pregnancy, childbirth, or breastfeeding. The virus can also be spread through blood transfusions or organ transplants if the blood or organs are infected. Without proper treatment, HIV can progress to acquired immunodeficiency syndrome (AIDS), which can lead to life-threatening complications. Due to the lack of a cure or vaccine, and the social stigma that often surrounds the disease, it continues to be a significant public health issue worldwide.

AIDS, or acquired immunodeficiency syndrome, is the final stage of HIV infection. It is characterized by a decline in the function of the immune system and the development of certain infections and cancers. AIDS is diagnosed when a person with HIV has a very low number of CD4+ T cells (also known as T helper cells), or when they develop certain opportunistic infections that take advantage of a weakened immune system.

When a person is infected with HIV, the virus begins to attack and kill CD4+ T cells, which are an important part of the immune system. Over time, the number of these cells in the body decreases, making the person more vulnerable to other infections. Without proper treatment, HIV can progress to AIDS (Hunt et al., 2008).

Proper antiretroviral therapy (ART) can slow or halt the progression of HIV to AIDS. ART involves taking a combination of medications that target different stages of the virus's life cycle. These drugs can reduce the amount of virus in the body, also known as the "viral load," to undetectable levels, and help to rebuild the immune system. ART is highly effective and can help people living with HIV lead long, healthy lives.

2.6 Zoonotic Transmission

Zoonotic transmission of HIV refers to the transfer of the virus from animals to humans. In the case of HIV, the virus is believed to have originated in primates, specifically chimpanzees and

sooty mangabey monkeys, and crossed over to humans through contact with infected blood or tissues, such as through hunting or butchering. The most widely accepted theory for the zoonotic origin of HIV is that it was transmitted to humans through the hunting and consumption of wild chimpanzees, gorillas or other primates, which have SIV (simian immunodeficiency virus) similar to HIV.

There is also a possibility that HIV may have crossed over to humans through bites from infected animals, or from contact with infected blood through hunting or butchering. However, this route of transmission is considered less likely, as the virus does not survive well outside the body and would have difficulty remaining infectious in the environment (Hemelaar, 2012).

It's also worth noting that while the origin of HIV-1, the most common and virulent form of the virus, is widely believed to be zoonotic, other strains of HIV have been identified that are likely the result of human-to-human transmission. It's important to mention that HIV is not a disease that can be transmitted from animals to humans under normal circumstances, and consumption of cooked meat, or any contact with animals and their byproducts, is safe and no pose any risk of HIV transmission.

2.7 Mode of Transmission

The most common mode of HIV transmission is through sexual contact, specifically through the exchange of bodily fluids such as blood, semen, vaginal fluids, and breast milk. This can happen during vaginal or anal sex, or less frequently through oral sex. The virus can also be transmitted through sharing needles or other injection equipment, as well as from mother to child during pregnancy, childbirth, or breastfeeding.

HIV can also be transmitted through blood transfusions or organ transplants if the blood or organs are infected, but this is relatively rare in countries with good screening procedures. It's also worth noting that HIV is not transmitted through casual contact, such as shaking hands, hugging, or sharing utensils with someone who has HIV. The virus cannot survive well outside of the body, and it's not airborne, it needs a direct access to bloodstream or body fluids for transmission (Gouws & Cuchi, 2012).

Preventive measures include practicing safe sex through the use of condoms, and avoidance of sharing needles, being tested for the virus regularly, and getting early treatment if diagnosed with HIV.

2.8 Mode of action of HIV replication

HIV replicates itself in the human body by targeting and entering certain cells of the immune system, specifically CD4+ T cells. These cells play an important role in the immune system by recognizing and responding to pathogens that enter the body.

Once the virus enters a CD4+ T cell, it uses an enzyme called reverse transcriptase to convert its genetic material, RNA, into DNA. This DNA is then integrated into the host cell's genome, where it can remain latent for long periods of time without producing new viral particles.

When the host cell becomes activated, the viral DNA is transcribed into viral RNA and new viral proteins are produced. These proteins then assemble with viral RNA and the viral enzymes, such as reverse transcriptase, protease and integrase to form new viral particles.

The new viral particles bud from the surface of the host cell, taking a piece of the cell's membrane with them. This process kills the host cell and releases the new viral particles into

the bloodstream, where they can infect other CD4+ T cells and continue the cycle of replication (Sauter & Kirchhoff, 2016).

It's worth noting that the immune system is constantly fighting against HIV replication and can slow it down by producing antiviral antibodies that bind to viral proteins, making it harder for the virus to infect new cells. The use of antiretroviral therapy (ART) helps in slowing down HIV replication by blocking the activity of reverse transcriptase and other viral enzymes, allowing the immune system to regain strength and fight the virus.

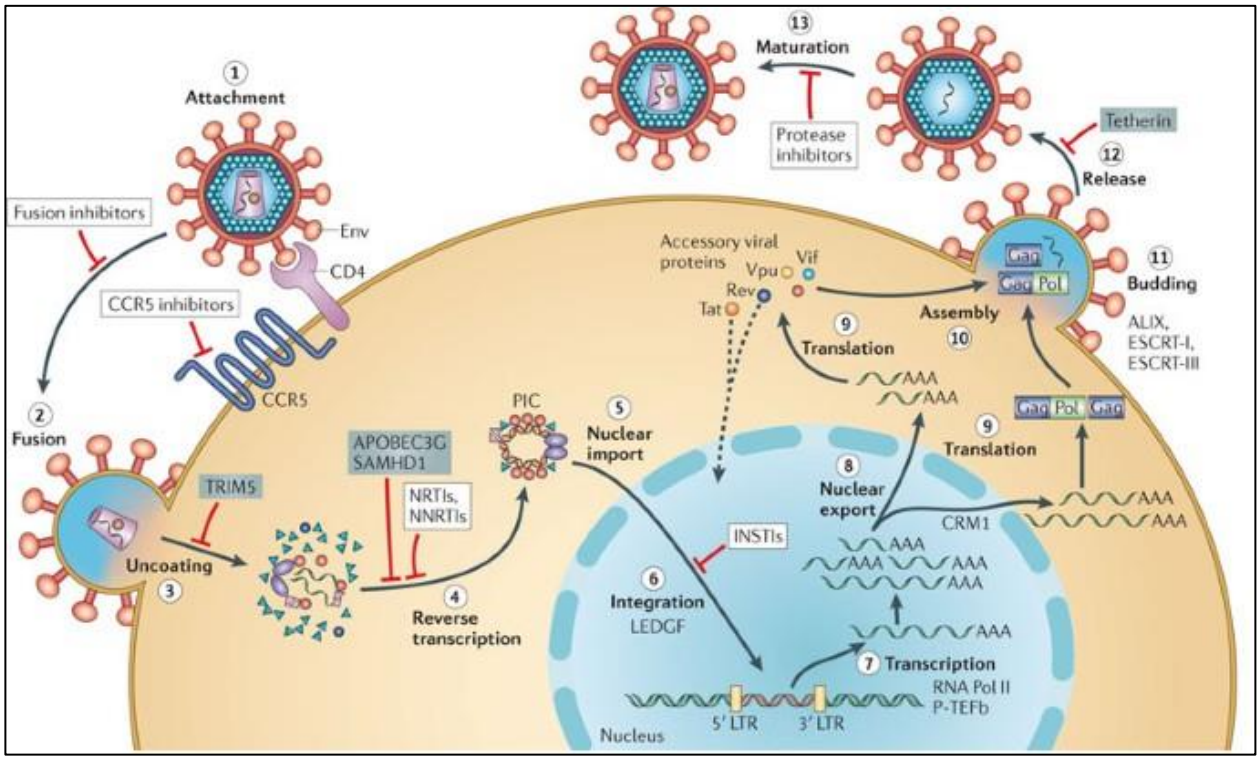

Figure 2.4 : *Infection cycle of HIV. The infection starts once the envelope (Env) glycoprotein spikes bind to the receptor CD4 and the membrane-spanning co-receptor CC-chemokine receptor 5 (CCR5) (step 1). This causes the viral and cellular membranes to fuse and the viral particle to enter the cell (step 2). Partially removing the core shell (step 3) makes it easier for reverse transcription (step 4), which in turn makes the pre-integration complex (PIC). After*

being brought into the nucleus of the cell (step 5), PIC-associated integrase helps the host chromatin-binding protein lens epithelium-derived growth factor (LEDGF) form the integrated provirus (step 6). Proviral transcription (step 7) is controlled by host RNA polymerase II (RNA Pol II) and positive transcription elongation factor b (P-TEFb). This makes viral mRNAs of different sizes, with the larger ones needing to leave the nucleus with the help of host protein CRM1 (step 8). mRNAs are used as blueprints for making proteins (step 9), and genome-length RNA is put into viral particles along with protein parts (step 10). ESCRT (endosomal sorting complex required for transport) complexes and ALIX help the viral particle break apart (step 11) and leave the cell (step 12). This is followed by protease-mediated maturation (step 13), which makes the viral particle infectious. Antiviral drugs could be used to stop HIV-1 at any point in its life cycle. The sites of action of clinical inhibitors (white boxes) and cellular restriction factors (blue boxes) are shown. INSTI stands for integrase strand transfer inhibitor. LTR stands for long terminal repeat. NNRTI stands for non-nucleoside reverse transcriptase inhibitor. (Engelman & Cherepanov, 2012)

2.9 Prevalence & Epidemiology of HIV around the globe

The prevalence and epidemiology of HIV around the globe vary widely by region and population. Globally, an estimated 38 million people were living with HIV at the end of 2019. Sub-Saharan Africa is the most affected region, with an estimated 25.8 million people living with HIV, accounting for two-thirds of the global total (Hemelaar et al., 2019). Other regions with high HIV prevalence include Eastern Europe and Central Asia, and Latin America.

In terms of transmission, sexual contact is the most common mode of HIV transmission worldwide, accounting for around 85% of new infections. Other modes of transmission include

sharing of needles and other injection equipment, and mother-to-child transmission during pregnancy, childbirth, or breastfeeding.

In terms of demographics, men who have sex with men and trans women are disproportionately affected by the epidemic, especially in countries with laws that criminalize or stigmatize homosexuality or gender identity. Other populations at higher risk of HIV infection include people who inject drugs, sex workers, and people living in poverty, as well as those in prison, refugee and migrant populations, and indigenous people (Viswasam et al., 2021). Overall, the epidemiology of HIV is a complex issue, and the disease disproportionately affects marginalized and vulnerable populations. Prevention and treatment programs that consider the specific needs and risk factors of these populations are needed to effectively address the HIV epidemic.

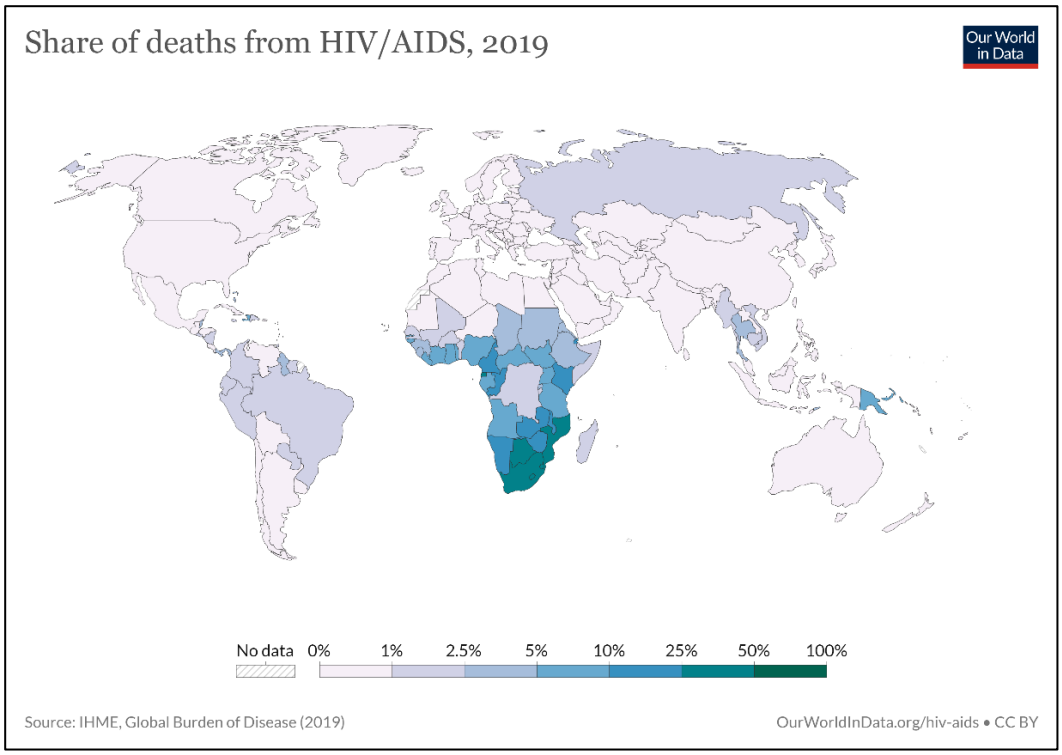

Figure 2.3 : *Number of deaths over the years around the globe-Our World in Data*

2.10 Epidemiology of HIV in Pakistan

The prevalence and epidemiology of HIV in Pakistan is relatively low compared to other countries in the region. According to the Joint United Nations Program on HIV/AIDS (UNAIDS), the estimated number of people living with HIV in Pakistan is 150,000 as of 2019, representing around 0.1% of the population.

The main mode of transmission of HIV in Pakistan is through the sharing of needles and other injection equipment by people who inject drugs. Other modes of transmission include heterosexual contact, and mother-to-child transmission. In Pakistan, the number of reported HIV cases are rising among men who have sex with men (MSM) and transgender people as well as Female Sex workers (FSWs) (Rabold et al., 2021).

In terms of demographics, most people living with HIV in Pakistan are men. However, there is a growing concern about the increasing number of women and children affected by the virus. Most cases have been reported from urban areas, and particularly from the cities such as Karachi, Lahore, and Islamabad

Overall, despite the relatively low overall prevalence of HIV in Pakistan, the country faces challenges in responding to the epidemic, particularly in terms of access to prevention and treatment services. There is also a need for addressing stigma and discrimination surrounding HIV/AIDS, particularly in marginalized communities such as men who have sex with men, sex workers and people who inject drugs.

2.11 Stages of HIV infected person

HIV infection is a progressive disease that occurs in three distinct stages: acute infection, chronic infection, and AIDS.

1. Acute HIV infection: This is the earliest stage of infection, occurring within a few weeks after exposure to the virus. During this stage, some people may experience flu-like symptoms such as fever, swollen lymph nodes, sore throat, fatigue, and rash. These symptoms usually disappear within several weeks, but the virus can still be spread to others. During this stage, the virus rapidly replicates in the body and the person's viral load is at its highest.
2. Chronic HIV infection: After the acute stage, the virus enters a phase of chronic infection, where the viral replication slows down, and the infected person may have no symptoms at all, or only mild symptoms. However, the virus continues to damage the immune system over time, resulting in a slow but steady decline in the number of CD4+ T cells, which are an important part of the immune system. This stage can last for several years or even decades, depending on the person.
3. AIDS: AIDS is the final stage of HIV infection, characterized by a severe decline in the function of the immune system. A person is diagnosed with AIDS when their CD4+ T cell count falls below 200 cells/mm3 or when they develop certain opportunistic infections that take advantage of a weakened immune system. These infections can include Pneumonia, Tuberculosis, and certain types of cancer such as Kaposi sarcoma. Without proper treatment, AIDS can be fatal (Prabhu, Harwell, & Kumarasamy, 2019).

It's worth noting that the course of HIV infection can vary widely among individuals, and some people may progress to AIDS faster than others. The use of antiretroviral therapy (ART) can slow or halt the progression of HIV to AIDS, allowing people living with HIV to lead long, healthy lives

2.12 Clinical Manifestations of HIV

The clinical manifestations of HIV can vary widely depending on the stage of the infection and the individual's overall health.

Acute HIV infection: During the early stage of infection, some people may experience flu-like symptoms such as fever, swollen lymph nodes, sore throat, fatigue, and rash within a few weeks of infection. This is known as acute HIV infection and symptoms usually disappear within several weeks, although the virus can still be spread to others (Sax, Bartlett, & Bloom, 2019).

Asymptomatic HIV infection: Many people who are infected with HIV may not have any symptoms for several years or even decades. During this time, the virus is still actively replicating and damaging the immune system, even though the person may feel healthy.

Symptomatic HIV infection: As the virus continues to replicate and the immune system becomes increasingly compromised, people with HIV may develop a variety of symptoms, including:

- Persistent fever
- Fatigue
- Unexplained weight loss
- Swollen lymph nodes
- Recurrent infections such as thrush or herpes simplex
- Diarrhea and other digestive problems
- Persistent dry cough
- Night sweats

- Skin rashes or discoloration
- Neurological symptoms such as confusion, memory loss, and difficulty walking

A person with HIV may progress to develop acquired immunodeficiency syndrome (AIDS) if they don't receive treatment, at this stage their immune system is severely damaged making them susceptible to certain life-threatening infections and cancers, also known as Opportunistic infections (Prabhu et al., 2019).

It is important to note that many of these symptoms can also be caused by other conditions, and not everyone with HIV will experience the same symptoms or progress to AIDS at the same rate (Sax et al., 2019). An HIV test and regular check-ups with a healthcare professional is the only way to confirm an HIV infection and monitor the progression of the disease.

2.13 Diagnosis of HIV

HIV can be diagnosed in several ways. The most common methods include:

- HIV Antibody tests: These tests look for antibodies that the body produces in response to an HIV infection. Antibodies are proteins that the body produces to fight off infections. These tests can be done using a sample of blood or oral fluid and can detect HIV infection within a few weeks of exposure.
- HIV Antigen/Antibody combination tests: These tests look for both antigens (proteins produced by the virus) and antibodies, they can detect HIV sooner than antibody-only tests, as early as 15-20 days post-infection (Bianchi et al., 2019; Fearon, 2005).
- Nucleic acid tests (NATs): These tests look for the genetic material of the virus itself and are generally considered the most accurate form of HIV testing but are also the most expensive.

These tests are used for very early HIV diagnosis or when there's a very high risk of exposure and can detect HIV as early as 9-11 days post-infection.

A positive result on an HIV test should be confirmed with a second test to rule out false positives. A negative result on an HIV test does not necessarily mean that a person is not infected with HIV, as the virus may not be detected by the test during the early stages of infection. It's also important to note that people who are at high risk of HIV should be tested regularly, regardless of whether they have symptoms, as early diagnosis and treatment can help to slow the progression of the disease and reduce the risk of transmission to others.

2.14 Preventive measures

There are several preventive measures that can help to reduce the risk of HIV infection. These include:

- Consistently and correctly using condoms: Using a new, lubricated condom every time you have sex can help to protect against HIV and other sexually transmitted infections.
- Pre-exposure prophylaxis (PrEP): PrEP is a medication that can be taken daily by people who are at high risk of HIV infection. When taken as prescribed, PrEP can reduce the risk of HIV infection by more than 90%.
- Post-exposure prophylaxis (PEP): PEP is a short course of antiretroviral drugs that can be taken after a high-risk exposure to HIV. It must be taken as soon as possible and within 72 hours after exposure to be effective and is meant to be used in emergency situations.
- Avoiding injection drug use: Sharing needles or other injection equipment can put you at a high risk of HIV infection. Not using drugs can help to reduce the risk of HIV infection, as well as other health problems associated with drug use.

- Treating other sexually transmitted infections: People who have other sexually transmitted infections such as syphilis or herpes are at a higher risk of getting HIV and can be more likely to spread it.
- Testing and early treatment: Knowing your HIV status and getting early treatment if you are infected can help to slow the progression of the disease and reduce the risk of transmission to others.
- Education and awareness: Education and awareness campaigns can help to reduce the stigma and discrimination associated with HIV and educate people on how to protect themselves and others.

It's worth noting that some of these preventive measures are more effective than others, and the best approach may vary depending on an individual's specific risk factors and circumstances (Hosek & Pettifor, 2019). Also worth mentioning that, as of 2020, there is a preventive HIV Vaccine that is under clinical trial and showing promising results, it may become available in the near future (Ng'uni, Chasara, & Ndhlovu, 2020).

2.15 Treatment of HIV

The treatment of HIV is done using antiretroviral therapy (ART), which is a combination of medications that work to slow or halt the replication of the virus (Thoueille, Choong, Cavassini, Buclin, & Decosterd, 2022). The goal of ART is to suppress the virus to undetectable levels and to maintain or restore the health of the immune system.

The main classes of drugs used in ART include:

- Nucleoside reverse transcriptase inhibitors (NRTIs): These drugs work by inhibiting the activity of reverse transcriptase, an enzyme that the virus needs to make copies of itself. Examples of NRTIs include zidovudine (AZT), lamivudine (3TC), and emtricitabine (FTC).
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs): These drugs also inhibit the activity of reverse transcriptase, but they work by binding to a different part of the enzyme than NRTIs. Examples of NNRTIs include nevirapine, efavirenz, and rilpivirine.
- Protease inhibitors (PIs): These drugs work by inhibiting the activity of protease, another enzyme that the virus needs to make copies of itself. Examples of PIs include atazanavir, darunavir, and ritonavir.
- Integrase inhibitors: These drugs work by inhibiting the activity of integrase, an enzyme the virus needs to insert its genetic material into the host cell DNA. Examples of Integrase inhibitors include raltegravir and dolutegravir.
- Entry inhibitors: These drugs work by preventing the virus from entering host cells. Examples of entry inhibitors include Maraviroc and Enfuvirtide.

However, when starting antiretroviral therapy (ART), it is generally recommended that people with HIV be prescribed a combination of at least three drugs from different classes, usually a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one of the other classes of drugs. This is known as “triple therapy" or "antiretroviral therapy" (ART).

The World Health Organization (WHO) recommends a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and one third drug from any of the remaining classes, this is called "preferred" or "recommended" ART regimens. This approach provides maximum viral suppression and prevents the development of drug resistance.

It's worth noting that the specific combination of drugs and the dosing regimen will vary depending on the patient's individual circumstances, including their current health status, any other medical conditions, and any prior exposure to antiretroviral medications. The use of fixed-dose combinations (FDCs) that contain multiple drugs in a single pill can also simplify treatment, improve adherence and lower the risk of drug resistance (Thoueille et al., 2022).

An important aspect of ART is close monitoring of the patient's treatment response, CD4+ count, viral load and other laboratory tests, as well as regularly monitoring for any potential side effects of the medication. The treatment will be adjusted or changed if necessary if no improvement or if the side effects are unacceptable. ART isn't a cure for HIV, but when taken correctly, it can effectively suppress the virus to undetectable levels, allowing people living with HIV to lead long, healthy lives.

2.16 Drug Resistance Mutations (DRMs)

Drug resistance mutations (DRMs) occur when the virus mutates in a way that allows it to continue replicating in the presence of antiretroviral therapy (ART). These mutations can occur in the genetic material of the virus and can result in changes to the proteins that are targeted by ART.

When a person is taking ART, the drugs work to suppress the replication of the virus. However, since the virus can replicate very rapidly and make mistakes in copying its genetic material, there is always a small chance that a mutation will occur that allows the virus to continue replicating in the presence of the drugs (Kagan, Dunn, Snell, Nettles, & Kaufman, 2019).

These drug-resistant viruses can reproduce and infect other cells, and if it happens to be the dominant viral strain in the body, it will be harder to suppress, making the treatment less effective and the virus can persist in the body.

There are several ways that DRMs can occur while a person is on ART:

- Poor adherence: When a person does not take their medications as prescribed, the virus can replicate and mutate more freely, increasing the risk of drug resistance.
- Mono/Dual therapy: If a person is taking only one or two drugs, the virus has a higher chance of developing resistance, as the drugs will be less effective in preventing the replication of the virus.
- Premature discontinuation: If a person stops taking ART before the virus is fully suppressed, the virus can continue to replicate, mutate, and select for drug-resistant strains.
- Late initiation: Starting ART when the virus has already progressed, and the immune system is damaged may not be as effective as starting early and may increase the risk of DRMs.

It's important to note that not everyone who is taking ART will develop DRMs, but to decrease the chances of it happening, it's crucial to take the medication as prescribed, regularly monitoring the virus and adjust the treatment regimen if necessary (Kagan et al., 2019). DRMs can also be identified through genotypic or Phenotypic testing, which can be used to guide treatment decisions and optimize outcomes.

The prevalence of DRMs in Pakistan is still considered to be low, however it is increasing over time (Abidi et al., 2022). There are several factors that can contribute to the development of DRMs in Pakistan, such as:

- Poor adherence: With lack of access to information and education, some people in Pakistan may not understand the importance of taking their ART as prescribed, which can increase the risk of DRMs.
- Mono/Dual therapy: Due to financial constraints, some people may not be able to afford triple therapy and are prescribed Mono/Dual therapy.
- Late initiation: Many people in Pakistan are not diagnosed with HIV until later stages of the disease, meaning that the virus has already progressed, and the immune system is damaged, making it more difficult to fully suppress the virus and decrease the risk of DRMs.
- Limited diagnostic capabilities: There's a lack of facilities for genotypic and Phenotypic testing to detect DRMs in Pakistan.
- Limited access to care: Many people living with HIV in Pakistan have limited access to HIV testing, care, and treatment, which can contribute to poor viral suppression, increased risk of DRMs, and poor health outcomes.

The government of Pakistan and international organizations are working to improve access to HIV testing, care, and treatment in the country. As well as education and awareness campaigns aimed to inform people about the importance of ART adherence, and the availability of treatment options. With the implementation of these strategies, it is expected that the number of people living with DRMs in Pakistan will decrease over time.

MATERIALS AND METHODS

3.1 Thesis Statement

No cross-sectional survey of HIV-1 has been done to date to provide a true depiction of the epidemiology in the nation especially targeting Punjab because it is needed to develop better public health policies related to the prevention and therapies, despite the fact that it may seem exhausting to invest in a disease that typically presents its fate, improperly tapping into the source of the disease can lead to its spread to high-risk groups. Additionally, it is necessary to alleviate the disease's morbidities, and genotyping can show which principal vector the virus used to propagate.

3.2 Ethics Statement

This research has been conducted in accordance with ethical principles and guidelines, including informed consent, confidentiality, and respect for participants. All data collected and analyzed in this study is kept strictly confidential and will only be used for the purposes of this thesis. Participants have the right to withdraw from the study at any time without any negative consequences. The findings of this research will be reported accurately and honestly, and any potential risks or harm to participants will be minimized.

3.3 Sampling

As the sampling criteria, Patients that tested positive for HIV and were receiving Antiretroviral therapy were selected in the study. However, the patients that were not taking any medications were excluded, despite being tested positive for the presence of the virus. This was done to ensure that drug resistant mutations are targeted.

3.4 Study Group

A total of 40 samples were included in the study to conduct the whole experimentation. This number of 40 totally satisfied the calculation for the cross-sectional epidemiological study of HIV.

$$Z = Z^2.p(1-p)/d^2$$

Whereas,

Z=Standard normal variate (for 5% error =1.96, ($p<0.05$)),

p= expected proportion of population's disease in previous studies; In Pakistan for HIV-1 it's 0.01

d= absolute error/precision

If we put the values in the given formula with 5% error rate ($p<0.05$), we got 12 & we got higher number of samples than this to strengthen this study. To conduct this cross-sectional study a certain group of patients included in this study, All the patients were HIV-1 positive and got their treatment from the Punjab AIDS control program, inclusion criteria is simple as this study mainly focus on DRMs, so this study includes that patients of HIV-1 who were on the treatment.

Sanger sequencing done for the identification of DRMs in HIV-1 positive samples, following steps done for this experimentation.

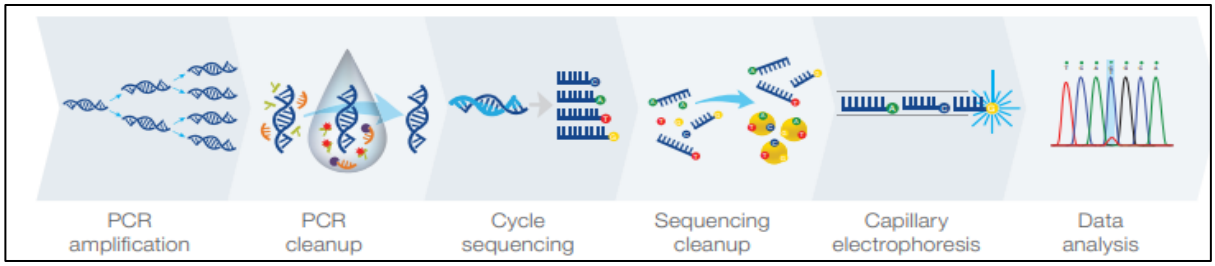

Figure 3.1 : *Flow Diagram of extraction of sequences by Sanger sequencing (Alidjinou et al., 2017).*

3.5 RNA Extraction from serum samples

There are a few steps involved in extracting viral RNA using the Qiagen viral RNA extraction kit (catalog no. 52904). Here is a general outline of the process:

- Collect the serum sample and centrifuge it to pellet the cells.
- Resuspend the cell pellet in lysis buffer (560ul) and incubate at room temperature for 5-10 minutes to lyse the cells and release the viral RNA.
- Add an equal volume of ethanol to the lysis mixture and mix well.
- Transfer the mixture to a QIAamp MinElute spin column that is placed in a 2 mL collection tube.
- Centrifuge the mixture for 1 minute at 8000 rpm to bind the RNA to the column.
- Discard the flow-through and place the spin column back into the same 2 mL collection tube.
- Add 500 µL of wash buffer 1 to the spin column and centrifuge for 1 minute at 8000 rpm to wash the RNA.
- Discard the flow-through and place the spin column back into the same 2 mL collection tube.
- Add 500 µL of wash buffer 2 to the spin column and centrifuge for 1 minute at maximum speed to wash the RNA.

- Discard the flow-through and place the spin column back into the same 2 mL collection tube.
- Centrifuge the spin column for an additional 1 minute at maximum speed to remove any remaining wash buffer.
- Place the spin column in a clean 1.5 mL microcentrifuge tube.
- Add 50-100 µL of RNase-free water to the center of the spin column matrix and allow the RNA to elute by capillary action.
- Centrifuge the spin column for an additional 1 minute at maximum speed to elute the RNA.
- The purified RNA is now ready for use in downstream applications.

3.6 cDNA Synthesis & Round 1 PCR

After the extraction of RNA from samples cDNA synthesized with the reverse primer (D). For this experimentation many primers were used for the maximum coverage of the *pol* region. A list of primers was given in the given table which were used in this experimentation. For this purpose, Qiagen OneStep RT-PCR kit (catalog no. 210212) used where cDNA and desired Round 1 product is being amplified by using pair of primers forward primer (A) & reverse primer (D) (10uM).

Virus	**Primer name**	**Primer Sequence 5' to 3'**	**Region in *pol***	**Direction**
	A	CAGGAGCAGA TGATACAG	Protease	Forward
	Bf	TGGACTGTCAA TGATATACA	Reverse Transcriptase	Forward

HIV-1	Br	TGTATATCATTGACAGTCCA	Reverse Transcriptase	Reverse
	CF	ACAGTGCAGGGGAAAGAA	Integrase	Forward
	Cr	TTCTTTCCCCTGCACTGT	Integrase	Reverse
	D	CCCTTCACCTTTCCAGAG	Integrase	Reverse
	a1	ATAGGGGGAATTGGAGGTTTTAT	Protease	Forward
	a2	AGGAATGGATGGCCCAAA	Protease	Forward
	b1	GGGTTATGAACTCCATCCTGATAAATGGAC	Reverse Transcriptase	Forward
	b2f	TGGAGAGCAATGGCTAGTGA	Reverse Transcriptase	Forward
	b2r	TCACTAGCCATTGCTCTCCA	Reverse Transcriptase	Reverse

Table 1 : *Primers details used for cDNA synthesis, round 1 & 2 PCR, and cycle sequencing PCR. All these 11 primers are used to amplify the maximum region of pol region in HIV-1 which is the most vulnerable region for DRMs (Nie, Detorio, & Schinazi, 2011).*

The recipe for the round 1 PCR master-mix is given below:

Round 1 PCR Recipe		
Sr. #	**Reagent**	**Quantity (1x)**
1	dH2O	27.75 ul
2	5x Reaction Buffer	10 ul
3	Enzyme Mix	2 ul
4	d NTPs	2 ul
5	RNase Inhibitor	0.25 ul
6	Primer For.	1.5 ul
7	Primer Rev.	1.5 ul
8	Template	5 ul
9	Total Volume	50 ul

Table 2 : *Recipe for the round 1 PCR master-mix*

Round 1 PCR Profile can also be seen in the table below:

Round 1 PCR profile		
Temp.	**Time**	**Cycles**
50°C	30 min	1x
94°C	5 min	1x

94°C	30 sec	40 x
49°C	30 sec	
68°C	2.5 min	
68°C	10 min	1x
10°C	hold/Infinity	Infinity

Table 3 : *Round 1 PCR Profile*

Round 2 PCR

For round 2 PCR 6 combinations were done for a single sample to cover maximum region with high quality score in sequencing. These six PCR combinations can be seen in the given table with their respective amplicon size.

Sr. No.	Primers Combination	Amplicon size (bp)
1	A-Br	994
2	Bf-Cr	1525
3	Cf-D	161
4	a2-b2r	1713
5	b2f-D	687
6	b1-Cr	1589

Table 4 : *Combination of Primers used in Round 2 PCR*

The recipe for the round 2 PCR master-mix is given below:

Round 2 PCR Recipe		
Sr. #	**Reagent**	**Quantity (1x)**
1	dH2O	27.75 ul
2	5x Reaction Buffer	10 ul
3	Enzyme Mix	2ul
4	d NTPs	2ul
5	RNase Inhibitor	0.25 ul
6	Primer For.	1.5 ul
7	Primer Rev.	1.5 ul
8	Template	5 ul
9	Total Volume	50 ul

Table 5 : *Recipe for the round 2 PCR master-mix*

Round 2 PCR Profile can also be seen in the table below:

Round 2 PCR profile		
Temp.	**Time**	**Cycles**
95 C	15 min	1x
94 C	30 sec	40 x

49 C	30 sec	
72 C	2: 30 sec	
72 C	7 min	1x
10 C	hold/Infinity	Infinity

Table 6 : *Round 1 PCR Profile*

3.7 Gel Electrophoresis

For tracking the PCR progress agarose gel is made for the visualizing of amplicons through gel electrophoresis experiment. 1.5% agarose gel made for this experiment, and it run on the Voltage of 110 for 45 minutes. To judge the exact size of amplicon 100bp Ladder (Invitrogen 100 bp Ladder Cat no. 15628019) run as a control.

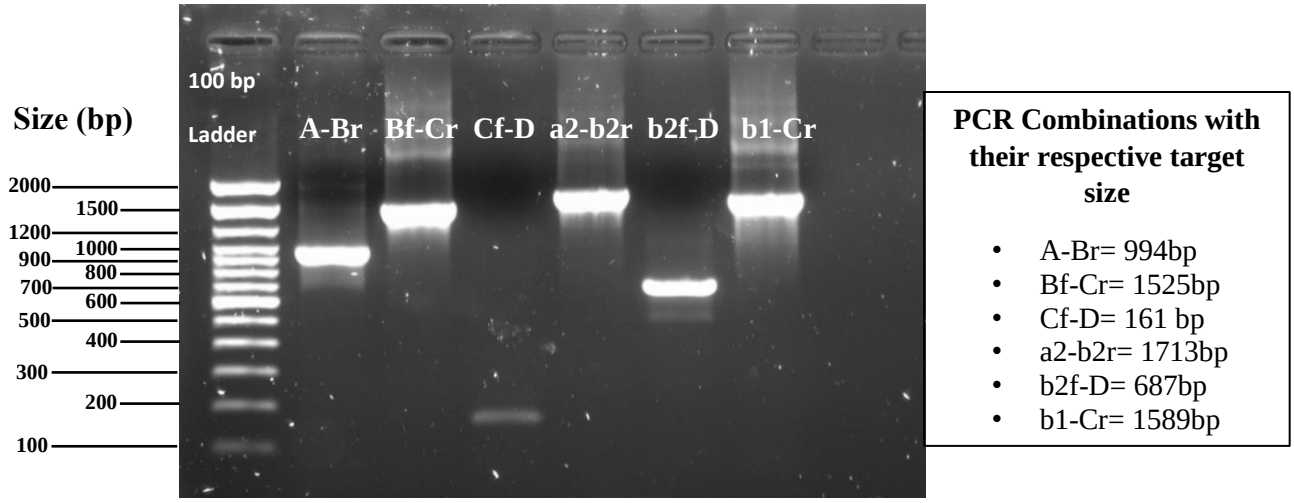

Figure 3.2 : *Gel Electrophoresis picture shows the amplified product of HIV-1 pol's region.*

3.8 PCR clean-up

Here is a protocol for purifying PCR products using calcium-coated magnetic beads:

- Mix the PCR product with a 1.8x volume of High Prep PCR binding beads solution (Cat no. AC-60050). For 45ul of PCR product 81ul of magnetic beads used.
- Place the tube in a magnetic stand to allow the beads to capture the PCR product.
- Carefully remove the supernatant, being careful not to disturb the beads.
- Wash the beads by adding 200ul of 80% ethanol and mixing gently.
- Carefully remove the ethanol, being careful not to disturb the beads.
- Repeat the washing step once more.
- To elute the purified PCR product, add a 40ul elution buffer or Nuclease free water to the beads and mix gently.
- Place the tube back in the magnetic stand to allow the beads to capture the elution buffer.
- Carefully remove the elution buffer or Nuclease free water, being careful not to disturb the beads.

3.9 Cycle Sequencing PCR

As the PCR products were purified by using HighPrep PCR clean up, these products were used as a template for the cycle sequencing PCR. For each PCR product a specific combination of primers was used for sequence that region. The following table shows the primers which were used to sequence that region.

Sr. No.	Primers Combination for PCR	Primers used to sequence for that region
1	A-Br	A, a1, a2, Br
2	Bf-Cr	Bf, b2r, b2f, Cr
3	Cf-D	Cf & D

4	a2-b2r	a2, b1, Br, Bf, b2r
5	b2f-D	b2f, Cr, Cf, D
6	b1-Cr	b1, Bf, b2r, b2f, Cr

Table 7 : *Six Primers combination used to cover the pol region, for each combination of primer multiple primers used to overlap the region for better sequencing results.*

Big Dye Terminator V3.1 Cycle Sequencing kit (Cat no. 4337455) used for this procedure & the following table shows the components used in the experiment.

Table 3.8: Reagents used in Cycle sequencing PCR.

Sr. No.	Component	Volume
1	Big dye	2 ul
2	Sequencing Buffer	2 ul
3	Primer	0.5 ul
4	Template	2 ul
5	dH2O	3.5 ul
6	Total volume	10 ul

Table 8 : *Reagents used in Cycle sequencing PCR.*

Cycle Sequencing PCR Profile is given below the table:

Cycle Sequencing PCR profile		
Temp.	**Time**	**Cycles**
94 C	20 sec	25 cycles
59 C	15 sec	
60 C	4 min	
4 C	hold/Infinity	hold/Infinity

Table 9 : *Recipe of Cycle sequencing PCR.*

3.10 Dye terminal purification

Protocol for the purification of cycle sequencing product is:

- Mix 10 µl of HighPrep DTR magnetic beads (DT-70050) with 10 µl of cycle sequencing product & gently mix it with pipette.
- Add 40ul of 85% ethanol and mix it well by up & down the pipette.
- Incubate the mixture at room temperature for 5 minutes.
- Place the tube in a magnetic separator and wait for the beads to separate from the liquid.
- Carefully remove the supernatant (liquid) and discard it.
- Wash the beads by adding 100 µl of wash solution (85% ethanol) to the tube and incubating at room temperature for 1 minute.
- Carefully remove the supernatant (liquid) and discard it.
- Repeat one more time to ensure that all contaminants have been removed.

- Elute the purified PCR product by adding 40 µl of elution buffer or Nuclease Free Water to the tube and incubating at room temperature for 1 minute.
- Place the tube in the magnetic separator and wait for the beads to separate from the liquid.
- Carefully remove the supernatant (liquid), which should contain the purified PCR product.

3.11 Capillary Electrophoresis

Purified cycle sequencing product is now ready for capillary electrophoresis, where in the end extracted sequence can be seen in the form of chromatograms. 40 ul of purified product poured in the 96-well plate & sealed with septa. This plate is now ready for the 3500XL Genetic Analyzer which was used for this procedure.

3.12 Data Analysis-Sequence editing

Sequencing files produced by 3500xl in the form of ABI files. This is the raw data which was trimmed by the Sequencher software to make a final FASTA file.

- Launch Sequencher and open the ABI files by going to File > Open > ABI File.
- Select the ABI files that you want to edit and click Open.
- The ABI files will be displayed in the Sequencher window.
- Use the trimming and editing tools in Sequencher to remove any low-quality or incorrect bases from the sequences. You can use the Trim tool to remove bases from the ends of sequences, or the Edit tool to make changes to the sequences.

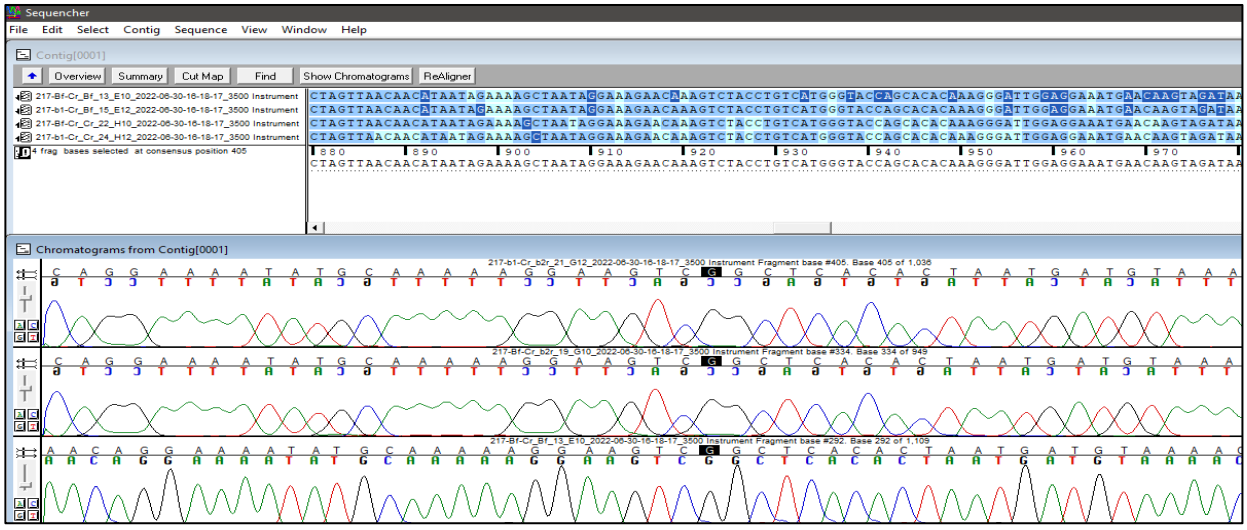

Figure 3.3 : *Sequencher 4.9 dashboard : Chromatograms for the editing of sample.*

- Once you have finished editing the sequences, you can export them as a consensus FASTA file by going to File > Export > Consensus FASTA.
- In the Export Consensus FASTA dialog box, select the sequences that you want to include in the FASTA file, and specify the output file name and location.
- Click Save to export the consensus FASTA file.

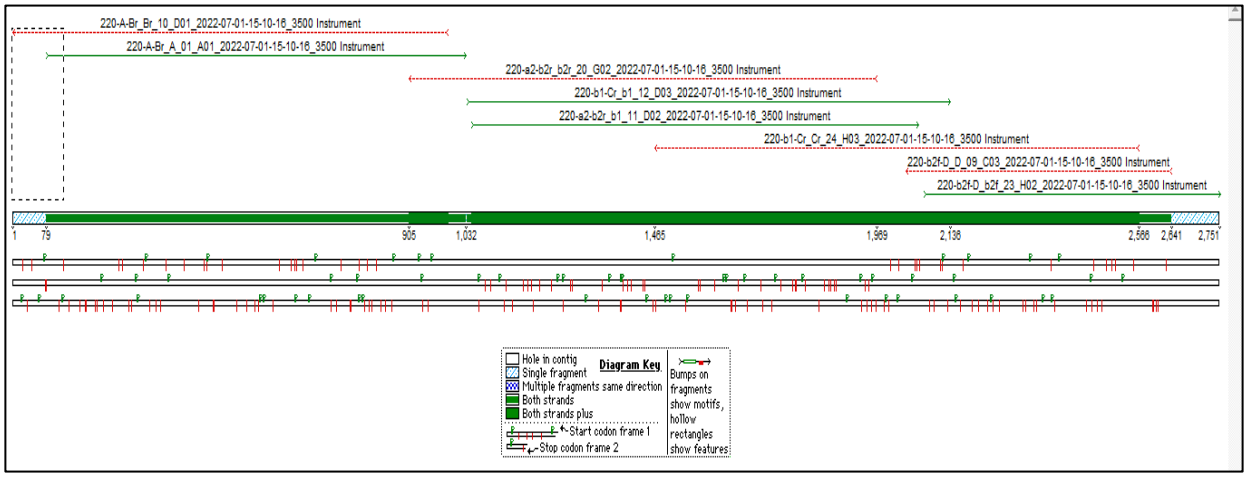

Figure 3.4 : *Primers overlapped in Sequencher 4.9 software covers the pol region of HIV-1.*

RESULTS

4.1 Sequence Alignment & phylogenetic tree

Sequences of additional HIV-1 subtypes were acquired in FASTA format from NCBI to evaluate the phylogenetic context of the sequenced samples. The additional sequences which were extracted from NCBI were:

- KC203330.1-KC203332.1 **(China)**
- KT581450.1 **(Germany)**
- JX227940.1 **(Senegal)**
- JX227940.1, KF890251.1, KF890250.1 **(India)**
- MT240851.1, MT240850.1, MT223668.1, MT223667.1, MT223665.1 **(Pakistan)**
- AY010480.1, FM164924.1 HIV-1 **(France)**
- L23102.1, FM164931.1 **(Taiwan)**

These all sequences include different subtypes which make a tree to validate previous study and it also strengthens this study. A rooted tree construct for this study with HBX2 as a reference sequence. For the construction of phylogenetic tree MEGA software used.

MEGA (Molecular Evolutionary Genetics Analysis) is a software package that can be used for both sequence alignment and phylogenetic tree construction.

To perform sequence alignment using MEGA, you can follow these steps:

- Open MEGA and select "Align" from the "Analysis" menu.
- Select the sequences you want to align and choose an alignment method (ClustalW).
- Adjust any additional settings as desired and click "OK" to start the alignment.

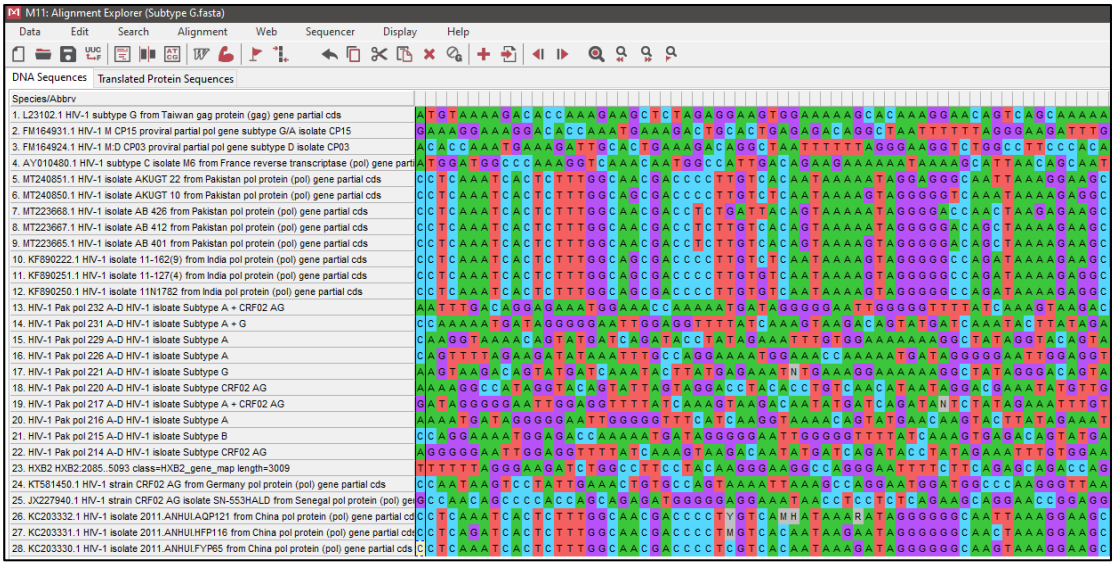

Figure 4.1: *Alignment of sequenced samples and retrieved sequences from NCBI database.*

Once you have the aligned sequences, you can then construct a phylogenetic tree using MEGA by following these steps:

- Open MEGA and select "Build" from the "Analysis" menu.
- Select the aligned sequences you want to use and choose a tree building method (Neighbor joining Method).
- Adjust any settings as per described and click "OK" to start the tree construction.

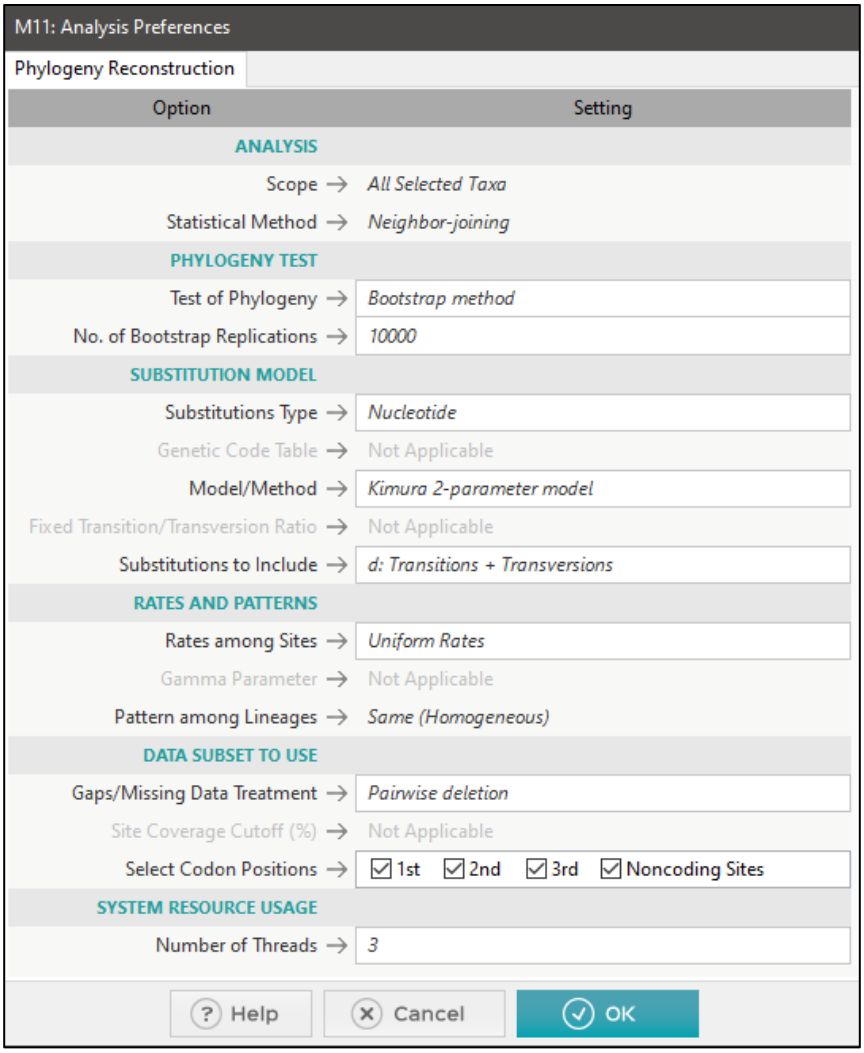

Figure 4.2 : *Parameters for the construction of Phylogenetic Tree using MEGAX software.*

By selecting these parameters, a phylogenetic tree was constructed which can be seen below.

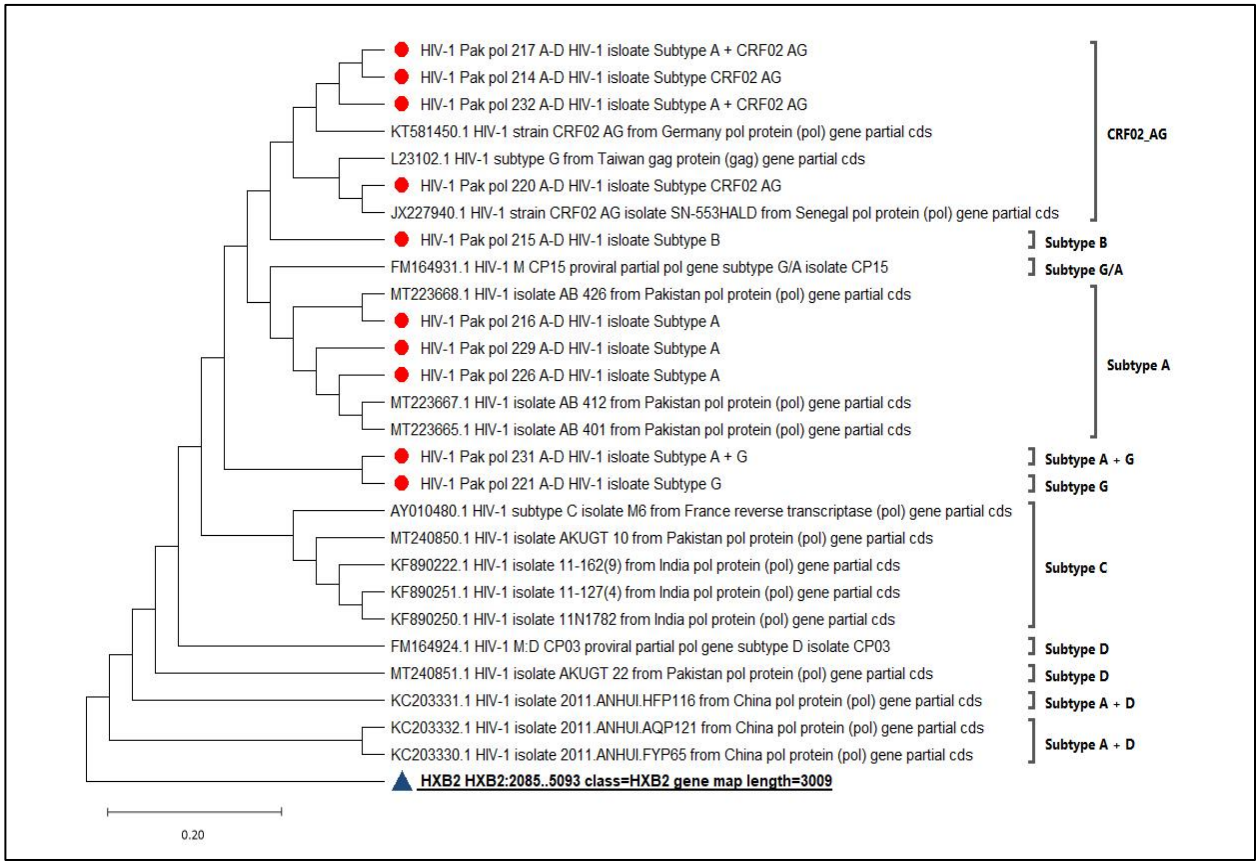

Figure 4.3 : *A Phylogenetic tree of 28 representative sequences of human immunodeficiency virus (HIV).*

This tree was generated using Neighbor Joining method in MEGAX. HXB2 using as the reference sequence, detected isolates of HIV are identified with red circle node markers (.). The distance scale represents the number of differences between the sequences.

4.2 Detection of Drug Resistance Mutations (DRMs) by "Stanford University HIV Drug resistance Database"

DRMs can be find by submitting sequences (FASTA file) in the "Stanford University HIV Drug resistance Database" where it's not only detects the DRMs but also characterize into its subtype & also finds the drugs that are resistance to the HIV virus (Shafer, 2006).

The Stanford HIV Drug Resistance Database (HIVDB) is an important tool for public health workers who are keeping track of ADR and TDR, scientists who are making new ARV drugs, and HIV care providers who are taking care of HIVDR patients. HIVDR researchers also use HIVDB to compare their findings to those of other studies and to do meta-analyses, which require data from many studies and make it possible to learn new things that can't be learned from just one survey.

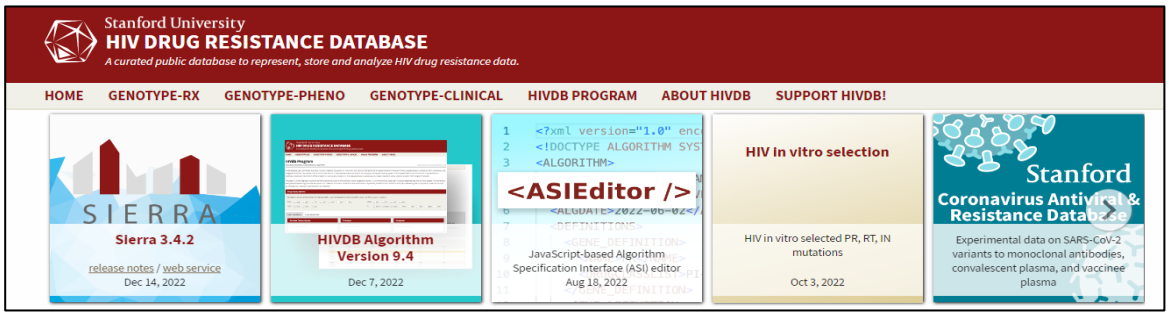

Figure 4.4 : *Stanford University HIV Drug resistance Database (HIVDB) Dashboard.*

To summarize the outcome in sequenced samples that makes a relation between drug resistance mutations with drugs can be seen in the R-plot given below.

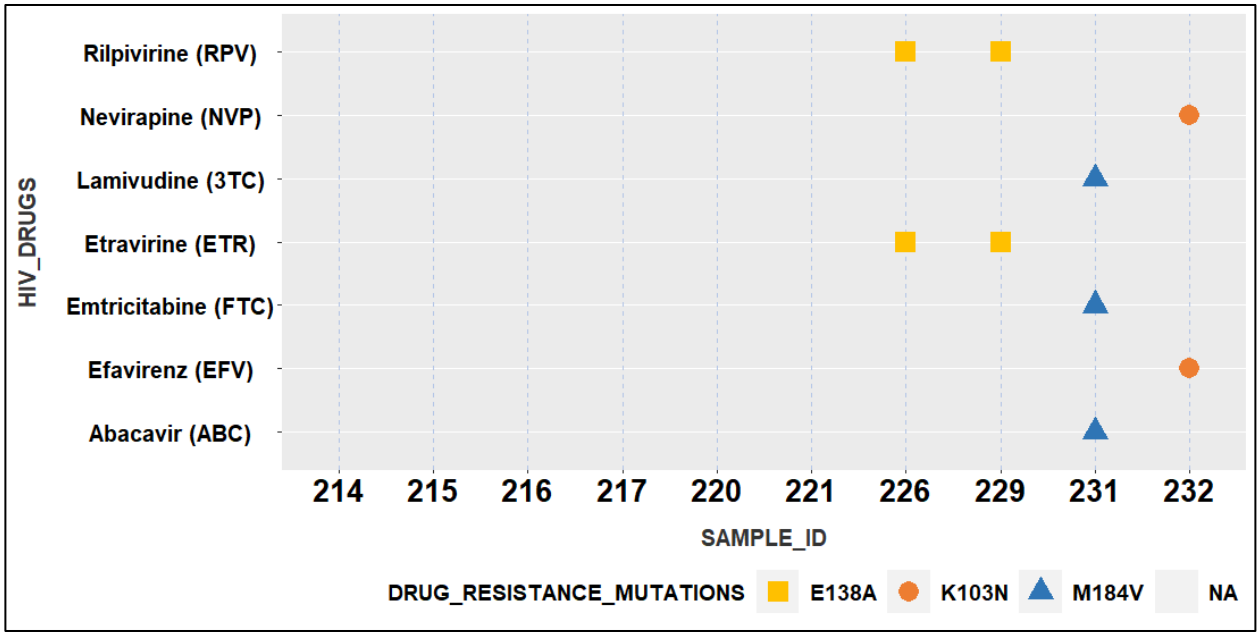

Figure 4.5 : *R-Plot shows the relation of 10 sequenced samples (x-axis) with drugs (y-axis) used against the HIV-1 Infected patients with their specific related mutation.*

4.2.1 Interpretation of above R-plot

In the above plot out of 10 sequenced samples 4 samples were resistance towards the following drugs as shown in the y-axis of the graph. All the drugs are the inhibitors for reverse transcriptase region in HIV-1. Relation of each mutation with its drug described below.

4.2.2 Sample_Ids 226 & 229 & E138A mutation

Rilpivirine (RPV) and Etravirine (ETR) are non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are used to treat HIV-1. The E138A mutation in HIV-1 is a resistance-associated mutation that can occur in the reverse transcriptase (RT) enzyme of the virus. This mutation can confer resistance to NNRTIs like RPV and ETR. This means that if a person's

HIV-1 strain has the E138A mutation, it may be less susceptible to the antiviral effects of RPV and ETR, making these drugs less effective in treating the virus(Sluis-Cremer et al., 2014).

4.2.3 Sample_Id 231 & M184V mutation

Lamivudine (3TC), emtricitabine (FTC) and abacavir (ABC) are nucleoside reverse transcriptase inhibitors (NRTIs) that are used to treat HIV-1. The M184V mutation in HIV-1 is a resistance-associated mutation that can occur in the reverse transcriptase (RT) enzyme of the virus. This mutation can confer resistance to NRTIs like 3TC, FTC and ABC, meaning that if a person's HIV-1 strain has the M184V mutation, it may be less susceptible to the antiviral effects of these drugs, making them less effective in treating the virus (Gallant, 2006). This is one of the most common mutations that happen in HIV-1 and is considered as a significant genetic marker for NRTI resistance.

4.2.4 Sample_Id 232 & K103N mutation

Nevirapine (NVP) and efavirenz (EFV) are non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are used to treat HIV-1. The K103N mutation in HIV-1 is a resistance-associated mutation that can occur in the reverse transcriptase (RT) enzyme of the virus. This mutation can confer resistance to NNRTIs like NVP and EFV, meaning that if a person's HIV-1 strain has the K103N mutation, it may be less susceptible to the antiviral effects of these drugs, making them less effective in treating the virus (Zhao et al., 2022). The K103N mutation is considered as one of the most common NNRTI resistance mutations and it is often associated with cross-resistance to other NNRTIs.

DISCUSSION

According to UNAIDS, people living with HIV in Pakistan is 210,000 [200,00-220,00] which is around 0.1% of the entire population. HIV is a significant public health issue in Pakistan, as it can lead to AIDS and death if left untreated. The disease disproportionately affects marginalized and at-risk populations, such as men who have sex with men, transgender people, and people who inject drugs. Additionally, there is a lack of awareness and stigma surrounding HIV in Pakistan, which can make it difficult for people to access testing and treatment. There are also limited resources and infrastructure in place to effectively respond to the HIV epidemic in the country. This combination of factors contributes to the ongoing spread of HIV in Pakistan and makes it a significant problem that needs to be addressed.

The number of patients infected by HIV is increasing day by day and moreover drug resistance mutations occurred as a problem in the process of elimination of AIDS in Pakistan. Drug resistance mutations can discourage the HIV elimination process in Pakistan by making it more difficult to effectively treat and control the spread of the virus. When HIV develops resistance to antiretroviral drugs, it becomes more difficult to suppress the virus and keep it at low levels in the body. This can lead to more serious health complications and an increased risk of transmission to others.

In Pakistan, where access to HIV treatment and resources may be limited, drug resistance mutations can also contribute to a lack of treatment options for people living with HIV. This can lead to poor health outcomes and increased transmission of the virus. Additionally, the cost of treating drug-resistant HIV can be higher than treating the virus with standard antiretroviral therapy, which can further discourage the HIV elimination process in Pakistan.

It's worth mentioning that the emergence of Drug resistance mutations can also be due to lack of adherence from patients, poor dispensation from the health care providers and lack of monitoring of the patient's response to the drugs.

Drug resistance mutations can occur when a person is exposed to high doses of a drug, or when a drug is not taken as prescribed. This can happen when a person takes more of a drug than they are supposed to, or when they take a drug that is not approved for their condition. Smuggling of drugs can also contribute to the development of drug resistance mutations, as the drugs may be of lower quality or have been tampered with.

In the case of HIV, the virus can quickly develop resistance to antiretroviral drugs if they are not taken as prescribed. This can happen when a person misses doses, takes the wrong drugs, or takes the drugs at the wrong time. Additionally, the HIV virus can also develop resistance to drugs when it is passed from person to person, as the virus can mutate to become resistant to the drugs used to treat it.

HIV is considered a taboo in Pakistan, as it is often associated with stigmatization and discrimination. This can be due to a lack of education and awareness about the virus, as well as societal attitudes towards people living with HIV. Many people living with HIV in Pakistan may face discrimination and marginalization in areas such as education, employment, and healthcare. This stigma and discrimination can make it difficult for people living with HIV to access appropriate healthcare, including testing and treatment. This can lead to poor health outcomes for people living with HIV and can also contribute to the spread of the virus. The taboo associated with HIV can also affect public health policy in Pakistan by making it difficult to implement effective HIV prevention and treatment programs. People may be less likely to get tested for HIV or seek treatment if they fear discrimination or stigmatization. This can

make it more difficult to control the spread of the virus and provide appropriate care for people living with HIV.

The Government of Pakistan has taken steps to address the issue of HIV and AIDS and is committed to reducing the spread of the virus and improving the lives of people living with HIV. However, more needs to be done to address the stigma and discrimination associated with HIV, and to ensure that people living with HIV have access to appropriate care and treatment.

In this study, HIV-1 positive serum samples collected from March 2022 to September 2022 for finding out the subtypes prevailed in Pakistan and investigate what kind of drugs shows resistance towards the recommended drugs for its treatment. To achieve these objectives Sanger dideoxy sequencing has been done to evaluate viral subtype and its DRMs.

In previous studies, **Subtype A, Subtype G** & **CRF_02AG** are the most prevailing subtypes of HIV-1 in Pakistan (Yaqub et al., 2019). In this study, 10 HIV samples sequenced & if we breakdown the samples according to their subtypes 3 are Subtype A, 3 are CRF_02AG, 2 are the Subtype G+A, 1 is subtype G & 1 is subtype G. The result of this study validates previous study and it also strengths the epidemiological study of HIV-1 in Pakistan. Mutational analysis of this study also strengthens the previous mutational studies of Pakistan (Shah et al., 2011). Different mutations are found out in this study using the Stanford HIVDB (https://hivdb.stanford.edu/). However, 3 mutations which give a strong resistance towards the Antiretroviral drugs (ARVs) find out in this study. All these mutations are linked with the reverse transcriptase gene in *pol* region. 2 out of 3 mutations that are resistance to reverse transcriptase inhibitors (NNRTI) are **K103A** & **E138A** that shows resistance towards efavirenz (EFV), nevirapine (NVP), etravirine (ETR) & rilpivirine (RPV) drugs. **M184V** is

another mutation that make drugs resistant to reverse transcriptase inhibitors (NRTI). This mutation makes drugs resistant to abacavir (ABC), emtricitabine (FTC), and lamivudine (3TC).

In the conclusionary remarks, Pakistan public health policy makers should modify the current drug regime because of the continuation of the finding such resistance mutations towards the aforementioned ARVs. In this study, all the participants are adults & following ARVs are given to people of this country according to National AIDS control Program (https://nacp.gov.pk/). In previous study M184V is the most common mutation prevailing in Pakistan (reference) ,not only in Pakistan one of our neighbor country India also got the most observed M184V mutation in 2007 (reference). However, ARV resistance mutation rate is low as compared to other countries in this region. This research to identify significant mutations by genotyping in Pakistani ARV-receiving individuals. We expect these mutations to proliferate soon according to this study. The results also show how important it is to keep an eye on resistance and use the best ARV combinations. Before beginning therapy for HIV-1, it is best to test for drug resistance. This will lead to better clinical outcomes. Primary care is still very important if Pakistan wants to reduce the number of people who need ARV therapy.

The future prospects of HIV in Pakistan are closely tied to the government's ability to address the issue. There is a need for increased investment in HIV prevention and treatment programs, as well as a focus on reducing stigma and discrimination towards those living with HIV. The increasing drug resistance mutations in HIV presents a major challenge but can be tackled through regular monitoring and effective management of antiretroviral therapy (ART) regimens.

The government can play a crucial role in addressing this issue by implementing comprehensive programs for HIV testing and counseling, promoting safe sexual practices, and providing access to ART for those who need it. Additionally, the government can work towards reducing the stigma and discrimination surrounding HIV/AIDS through awareness campaigns and education initiatives. Finally, the government

Anti-Retro Viral Drugs (Adult)- List of Pharmaceuticals and Health Items				
Sr. No.	**Product Name**	**Dose**	**Pack Size**	**Comments**
1	Efavirenz + (Lamivudine+ Zidovudine) Tablet	(150+300) +600mg	90Tab Bottle	Adult-First Line
2	Lamivudine + Nevirapine +Zidovudine Tablet	150+200+300mg	60Tab Bottle	Adult-First Line
3	Efavirenz+Lamivudine+Tenofovir Tablet	600+300+300mg	30Tab Bottle	Adult-First Line
4	Lamivudine/Tenofovir Tablet	300+300mg	30Tab Bottle	Adult-First Line
5	Dolutegravir/Lamivudine/Tenofovir	50/300/300mg	30Tab Bottle	Adult-First Line

6	Zidovudine Tablet	300mg	60Tab Bottle	Single Dose First Line
7	Lamivudine Tablet	150mg	60Tab Bottle	Single Dose First Line
8	Nevirapine Tablet	200mg	60Tab Bottle	Single Dose First Line
9	Efavirenz Tablet	600mg	30Tab Bottle	Single Dose First Line
10	Tenofovir tablets	300mg	30Tab Bottle	Single Dose First Line
11	Lopinavir 200mg + Ritonavir 50mg	200mg/50mg	120 Tab Bottle	Adult Second line
12	Raltegravir Tablet	400mg	60Tab Bottle	Adult Third Line
13	Abacavir Tablet	300mg	60Tab Bottle	Adult First Line

14	Dolutegravir Tablet	50 mg	60Tab Bottle	Adult Third line

Table 10 : *List of Pharmaceutical drugs (ARVs) used by National AIDS Control Program (NACP) for Adults in Pakistan. Highlighted drugs are those drugs which were identified as resistance towards the mutated HIV in this study.*

can also collaborate with civil society organizations and communities affected by HIV to provide support and resources for those living with the disease. By working together, the government and the wider society can help to mitigate the impact of HIV in Pakistan and improve the prospects for those living with the disease.

CONCLUSIONS AND FUTURE PROSPECTS

The conclusion of this research could highlight the high prevalence of the E138A, K103N, and M184V mutations in the Pakistani population infected with HIV-1, indicating a need for increased vigilance in the monitoring and management of the disease. Additionally, these findings could emphasize the importance of early detection of drug-resistant mutations and the selection of appropriate antiretroviral therapy regimens to ensure effective treatment and prevent the spread of drug-resistant strains.

Regarding future prospects, this research could inform the development of public health policies aimed at reducing the spread of drug-resistant HIV-1 in Pakistan. For example, these findings could support the implementation of guidelines for regular genetic testing of HIV-1 infected individuals, as well as the creation of surveillance programs to monitor the emergence of new drug-resistant strains.

This study could also stimulate further research into the genetic diversity of HIV-1 in Pakistan and its impact on treatment outcomes, providing valuable insights into the challenges facing healthcare professionals and public health policymakers in the fight against the disease.

REFERENCES

Abidi, S. H., Nduva, G. M., Siddiqui, D., Rafaqat, W., Mahmood, S. F., Siddiqui, A. R., . . . Memon, S. (2021). Phylogenetic and drug-resistance analysis of HIV-1 sequences from an extensive paediatric HIV-1 outbreak in Larkana, Pakistan. *Frontiers in microbiology, 12*, 658186.

Abidi, S. H., Siddiqui, D., Mahmood, S. F., Siddiqui, A. R., Nathwani, A. A., Hotwani, A., . . . Mir, F. (2022). Unassigned complex unique recombinant forms related to CRF36_cpx in children identified in an HIV-1 outbreak in Pakistan. *AIDS research and human retroviruses, 38*(10), 806-811.

Alidjinou, E., Deldalle, J., Hallaert, C., Robineau, O., Ajana, F., Choisy, P., . . . Bocket, L. (2017). RNA and DNA Sanger sequencing versus next-generation sequencing for HIV-1 drug resistance testing in treatment-naive patients. *Journal of Antimicrobial Chemotherapy, 72*(10), 2823-2830.

Bbosa, N., Kaleebu, P., & Ssemwanga, D. (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS, 14*(3), 153-160.

Bianchi, F., Cohn, J., Sacks, E., Bailey, R., Lemaire, J.-F., Machekano, R., . . . Odhiambo, C. O. (2019). Evaluation of a routine point-of-care intervention for early infant diagnosis of HIV: an observational study in eight African countries. *The lancet HIV, 6*(6), e373-e381.

Drylewicz, J., Matheron, S., Lazaro, E., Damond, F., Bonnet, F., Simon, F., . . . Thiébaut, R. (2008). Comparison of viro-immunological marker changes between HIV-1 and HIV-2-infected patients in France. *Aids, 22*(4), 457-468.

Eberle, J., & Gürtler, L. (2012). HIV types, groups, subtypes and recombinant forms: errors in replication, selection pressure and quasispecies. *Intervirology, 55*(2), 79-83.

Engelman, A., & Cherepanov, P. (2012). The structural biology of HIV-1: mechanistic and therapeutic insights. *Nature Reviews Microbiology, 10*(4), 279-290.

Fearon, M. (2005). The laboratory diagnosis of HIV infections. *Canadian Journal of Infectious Diseases and Medical Microbiology, 16*(1), 26-30.

Gallant, J. E. (2006). The M184V mutation: what it does, how to prevent it, and what to do with it when it's there. *AIDS READER-NEW YORK-, 16*(10), 556.

Gouws, E., & Cuchi, P. (2012). Focusing the HIV response through estimating the major modes of HIV transmission: a multi-country analysis. *Sexually transmitted infections, 88*(Suppl 2), i76-i85.

Gregson, J., Tang, M., Ndembi, N., Hamers, R. L., Rhee, S.-Y., Marconi, V. C., . . . de Wit, T. R. (2016). Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *The Lancet infectious diseases, 16*(5), 565-575.

Hemelaar, J. (2012). The origin and diversity of the HIV-1 pandemic. *Trends in molecular medicine, 18*(3), 182-192.

Hemelaar, J., Elangovan, R., Yun, J., Dickson-Tetteh, L., Fleminger, I., Kirtley, S., . . . Alash'le G, A. (2019). Global and regional molecular epidemiology of HIV-1, 1990–2015: a systematic review, global survey, and trend analysis. *The Lancet infectious diseases, 19*(2), 143-155.

Hosek, S., & Pettifor, A. (2019). HIV prevention interventions for adolescents. *Current HIV/AIDS Reports, 16*, 120-128.

Hunt, P. W., Brenchley, J., Sinclair, E., McCune, J. M., Roland, M., Shafer, K. P., . . . Lampiris, H. (2008). Relationship between T cell activation and CD4+ T cell count in HIV-

seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *The Journal of infectious diseases, 197*(1), 126-133.

Kagan, R. M., Dunn, K. J., Snell, G. P., Nettles, R. E., & Kaufman, H. W. (2019). Trends in HIV-1 drug resistance mutations from a US reference laboratory from 2006 to 2017. *AIDS research and human retroviruses, 35*(8), 698-709.

Kilmarx, P. H. (2009). Global epidemiology of HIV. *Current Opinion in HIV and AIDS, 4*(4), 240-246.

Ng'uni, T., Chasara, C., & Ndhlovu, Z. M. (2020). Major scientific hurdles in HIV vaccine development: historical perspective and future directions. *Frontiers in immunology, 11*, 590780.

Nie, T., Detorio, M., & Schinazi, R. F. (2011). Universal profiling of HIV-1 pol for genotypic study and resistance analysis across subtypes. *Antiviral therapy, 16*(8), 1267-1275.

Prabhu, S., Harwell, J. I., & Kumarasamy, N. (2019). Advanced HIV: diagnosis, treatment, and prevention. *The lancet HIV, 6*(8), e540-e551.

Rabold, E. M., Ali, H., Fernandez, D., Knuth, M., Schenkel, K., Asghar, R. J., . . . Morgan, O. (2021). Systematic review of reported HIV outbreaks, Pakistan, 2000–2019. *Emerging infectious diseases, 27*(4), 1039.

Sauter, D., & Kirchhoff, F. (2016). HIV replication: a game of hide and sense. *Current Opinion in HIV and AIDS, 11*(2), 173-181.

Sax, P. E., Bartlett, J., & Bloom, A. (2019). Acute and early HIV infection: Clinical manifestations and diagnosis. *UpToDate [Internet]*.

Schipani-McLaughlin, A. M., Lambert, D., Lauckner, C., & Hansen, N. (2017). HIV/AIDS: the last 30-plus years. *HIV/AIDS in rural communities: Research, education, and advocacy*, 33-44.

Shafer, R. W. (2006). Rationale and uses of a public HIV drug-resistance database. *The Journal of infectious diseases, 194*(Supplement_1), S51-S58.

Shah, S., Xing, H., Altaf, A., Chen, B., Liao, L., Jia, Y., . . . Shao, Y. (2011). Antiretroviral drug resistance mutations among treated and treatment-naive patients in Pakistan: diversity of the HIV type 1 pol gene in Pakistan. *AIDS research and human retroviruses, 27*(12), 1277-1282.

Sluis-Cremer, N., Jordan, M. R., Huber, K., Wallis, C. L., Bertagnolio, S., Mellors, J. W., . . . Harrigan, P. R. (2014). E138A in HIV-1 reverse transcriptase is more common in subtype C than B: implications for rilpivirine use in resource-limited settings. *Antiviral research, 107*, 31-34.

Stoff, D. M., Khalsa, J. H., Monjan, A., & Portegies, P. (2004). Introduction: HIV/AIDS and aging. In (Vol. 18, pp. 1-2): LWW.

Thoueille, P., Choong, E., Cavassini, M., Buclin, T., & Decosterd, L. A. (2022). Long-acting antiretrovirals: a new era for the management and prevention of HIV infection. *Journal of Antimicrobial Chemotherapy, 77*(2), 290-302.

van Heuvel, Y., Schatz, S., Rosengarten, J. F., & Stitz, J. (2022). Infectious RNA: Human Immunodeficiency Virus (HIV) Biology, Therapeutic Intervention, and the Quest for a Vaccine. *Toxins, 14*(2), 138.

Viswasam, N., Rivera, J., Comins, C., Rao, A., Lyons, C. E., & Baral, S. (2021). The Epidemiology of HIV Among Sex Workers Around the World: Implications for

Research, Programmes, and Policy. *Sex Work, Health, and Human Rights: Global Inequities, Challenges, and Opportunities for Action*, 15-39.

Volberding, P. A., & Deeks, S. G. (2010). Antiretroviral therapy and management of HIV infection. *The Lancet, 376*(9734), 49-62.

Weber, I. T., Wang, Y.-F., & Harrison, R. W. (2021). HIV protease: Historical perspective and current research. *Viruses, 13*(5), 839.

Yaqub, S., Yaqub, T., Shabbir, M. Z., Nadeem, A., Mukhtar, N., Rehman, F. A. U., . . . Sohail, T. (2019). Circulation of multiple subtypes (A, G and CRFs 02_AG) of human immunodeficiency virus type 1 (HIV-1) in selected districts of Punjab province, Pakistan. *Archives of Virology, 164*, 3081-3087.

Zhao, B., Song, W., Kang, M., Dong, X., Li, X., Wang, L., . . . Wang, L. (2022). Molecular network analysis reveals transmission of HIV-1 drug-resistant strains among newly diagnosed HIV-1 infections in a moderately HIV Endemic City in China. *Frontiers in microbiology, 12*, 4061.

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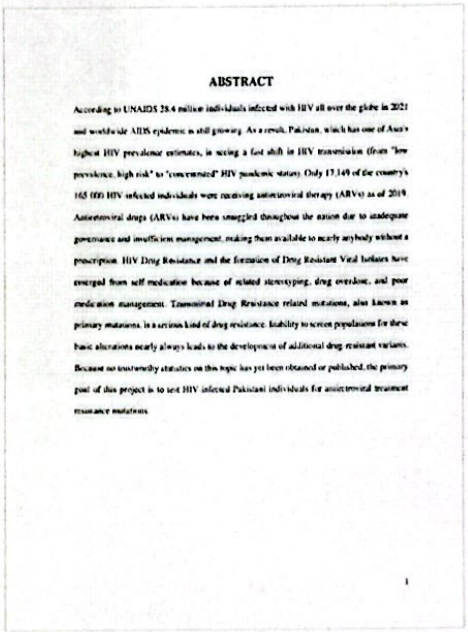

ABSTRACT

According to UNAIDS 28.4 million individuals infected with HIV all over the globe in 2021 and worldwide AIDS epidemic is still growing. As a result, Pakistan, which has one of Asia's highest HIV prevalence estimates, is seeing a fast shift in HIV transmission (from "low prevalence, high risk" to "concentrated" HIV pandemic status). Only 17,149 of the country's 165 000 HIV infected individuals were receiving antiretroviral therapy (ARVs) as of 2019. Antiretroviral drugs (ARVs) have been smuggled throughout the nation due to inadequate governance and insufficient management, making them available to nearly anybody without a prescription. HIV Drug Resistance and the formation of Drug Resistant Viral Isolates have emerged from self medication because of related stereotyping, drug overdose, and poor medication management. Transmitted Drug Resistance related mutations, also known as primary mutations, is a serious kind of drug resistance. Inability to screen populations for these basic alterations nearly always leads to the development of additional drug resistant variants. Because no trustworthy statistics on this topic has yet been obtained or published, the primary goal of this project is to test HIV infected Pakistani individuals for antiretroviral treatment resistance mutations.

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