Assessment of Mitochondrial DNA Damage Among HIV-Positive Teenagers in South West Nigeria

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Certification

This is to certify that **Kordinum Alumona** with matriculation number LCU/PG/001932 carried out this research work titled "Assessment of Mitochondrial DNA Damage Among HIV-Positive Teenagers in Southwest Nigeria." in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master Degree (M.Sc.) in Molecular Biology and Genomics and that this has not been previously submitted.

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Dedication

This research work is dedicated to the Almighty God for making its completion possible**.**

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Abstract

HIV/AIDS is one of the most lethal infectious diseases in the world, particularly in Sub-Saharan Africa, where it has significantly impacted health outcomes and life expectancy. This study focused on how HIV and highly active antiretroviral therapy (HAART) affects adolescent mitochondrial DNA (mtDNA). Previous research has shown that HIV indirectly reduces the quantities of mitochondrial DNA in cells through apoptosis during infection and treatment, and may induce genomic instability. This study aims to determine and compare mtDNA copy numbers and deletion levels among HIV-positive adolescents compared to HIV-negative adolescents. This study also aims to determine the level of genomic instability in HIV-positive adolescents. This pilot study utilized established real-time polymerase chain reaction (qPCR) protocols to determine the mtDNA copy numbers and damage, measuring the mtDNA ND1 and ND4 genes and the human nuclear B2M gene. The research population comprised 30 adolescents living with HIV on HAART and 30 HIV-negative adolescents recruited from the Nigerian Institute of Medical Researchers HAART clinic and University College Hospital Ibadan, respectively. We found a higher mitochondrial copy number in HIV-positive adolescents (mean=87.87±1.62) than in HIV-negative adolescents (mean =53.18±30.52; p-value=<0.05). These higher mitochondrial DNA copy numbers in positive HIV adolescents could be due to the early start-up of antiretroviral therapy and the body repair mechanisms working more to replace affected mtDNA. Also, mtDNA deletion level was lower among HIV-positive adolescents (mean=25.84±3.96) compared to the HIV-negative adolescents (mean =35.26±9.55; p-value=<0.05). Further studies should elucidate why mtDNA copy number is higher among HIV-positive adolescents receiving HAART and its impact on genome stability.

Keywords: Adolescents, Human immune deficiency virus (HIV), Mitochondrial DNA, Antiretroviral therapy

Word Count: 254

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List of Acronyms

Abbreviation	Meaning
mtDNA	Mitochondrial DNA
HIV	Human Immunodeficiency Virus
OXPHOS	Oxidative Phosphorylation
DNA	Deoxyribonucleic Acid
RC	Respiratory Chain
IMM	Inner Membrane
RNA	Ribonucleic Acid
Trna	Transfer Ribonucleic Acid
dsDNA	Double-stranded DNA
ART	Antiretroviral Therapy
CA	Capsid Protein
NC	Nucleocapsid
LRT	Long Terminal Repeats
RT	Reverse Transcriptase
SU	Surface Protein
TM	Transmembrane Protein
RNA	Ribonucleic Acid
Env	Viral Envelope
PR	Protease
AIDS	Acquired Immunodeficiency Syndrome
NRTI	Nucleoside Reverse Transcriptase
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
INSTIs	Integrase Strand Transfer Inhibitors
PI	Protease Inhibitors
FI	Fusion Inhibitors
FDA	Food and Drug Administration
ATP	Adenosine Triphosphate
SIV	Simian Immunodeficiency Virus

Abbreviation	Meaning
HAART	Highly Active Antiretroviral Therapy
POLRMT	RNA Polymerase
OH	Origin of Heavy Strand
OL	Origin of Light Strand
CBS	Conserved Sequence Blocks
ΔΨm	Membrane Potential
PLHIV	People Living With HIV
VPR.	Viral Protein
PCR.	Polymerase Chain Reaction
GP	Glycoprotein
TP	Typical Progressors
TNF	Tumor Necrosis Factor
ROS	Reactive Oxygen Species
MMX	Master Mix

Chapter One

Introduction

1.1 Background of study

The human immunodeficiency virus (HIV) is one of the world's most lethal infectious diseases, especially in Sub-Saharan Africa. It has influenced health outcomes and life expectancy in recent decades. The human immunodeficiency virus (HIV) is a member of the Lentivirus genus, a subfamily of the Retroviridae family[1]. It belongs to the lentivirus family, and there are two types of lentiviruses; Type 1 and Type 2 (HIV-1 and HIV-2) [2]. They are distinguished by genetic makeup and viral antigens (HIV-1 and HIV-2). It is believed to have originated from non-human primates and was transmitted through the process of Zoonosis. HIV-1 originated in southern Cameroon from wild chimpanzees infected with simian immunodeficiency virus (SIV)[3]. HIV-2 originated from sooty mangabeys, also carriers of the simian immunodeficiency virus and found in West Africa. The most common type of HIV is HIV-1. HIV-1 is quite virulent and infectious; however, HIV-2 is most prevalent in West Africa and has reduced virulence and infectivity. Because of this, signs of infection are typically not recognized until after pre-exposure[4,5]. Simian Immunodeficiency non-human primate virus is grouped under the lentivirus genus like HIV. Phylogenic analysis shows that HIV was introduced into the human population in the year 1920 – 1940[2].

Seven key phases comprise the HIV lifecycle: binding, fusion, reverse transcription, integration, replication, assembly, and budding (Appendix IX). The mechanism of HIV infection is best defined as an opportunistic, purposeful manipulation of host cells, causing significant dysregulation of the human immune system, including intrinsic, innate, and adaptive immunity, as well as several nonimmune cells and tissues[6].

Step 1 of the HIV infection process involves the interaction of glycoproteins on the surface of the HIV virus, such as Env/gp120, with CD4 and CCR5/CXCR4 receptors on the surface of a target CD4 T cell. Step 1 is blocked by coreceptor antagonists. This makes it easier for the HIV membrane to fuse with the cell membrane (step 2). Fusion inhibitors stop this process in its halt. HIV reverse transcriptase converts viral RNA into DNA in step three when viral capsids carrying HIV enzymes and viral RNA are released into the cell cytoplasm. HIV reverse transcriptase is blocked by nucleoside-analog and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs). Once within the cell nucleus, HIV integrase and transcription combine the viral DNA with the host genome (steps 5 and 6). INIs and INSTIs are integrase inhibitors that block the integration stage. After the transcribed DNA has been released into the cytoplasm for translation via Rev-mediated export (steps 7 and 8), assembly into the viral capsid (steps 9 and 10), and release (step 10), HIV protease cleaves polyproteins to produce an active virus that may infect new cells. Protease inhibitors stop the final release of a virus that is capable of reproducing. This can be seen in appendices IX.[22]

HIV infection is characterized by a gradual immune system deterioration brought on by a significant decrease in CD4 T cells. HIV infection causes an abnormal inflammatory response that leads to the loss of CD4 T lymphocytes, and HIV also employs cells for viral integration, replication, and release during viral pathogenesis[7-9]. Proinflammatory cytokines are also markedly upregulated by HIV infection, which accelerates the pathogenesis of the virus[10–13]. The development of reservoirs and viral latency are two main obstacles to HIV treatment. The virus hides in reservoirs during latency, a reversible inactive stage in which it continues to exist in host cells but does not reproduce[14]. Target cells, such as CD4 T cells, macrophages, and lymphoid

tissues, are infected by HIV, which then integrates as proviral DNA into the host genome to create reservoirs. The integration of proviral DNA causes genomic instabilities, by causing cellular damage. which might lead to damage in the nuclear and mitochondrial DNA and eventually trigger the development of cancer. Antiretroviral therapy (ART) enables virological control of HIV replication and prevents CD4 T cell counts from dropping below the threshold (200 cells/L) associated with severe immune deficiency; however, ART cessation readily permits activation of reservoir-residing cells and the production of actively replicating HIV[11,15-18].

Highly active antiretroviral therapy, often known as HAART, is now the most popular method of HIV treatment[19,20]. When ART was first developed, medications were administered as monotherapies; however, the rising prevalence of drug resistance prompted the introduction of combination therapy to reduce HIV-related morbidity and death and effectively limit viral replication[21]. Although ART is very effective at lowering the amount of actively replicating virus, once ART is discontinued, viremia returns to levels comparable to those seen before ART therapy[22]. HIV reservoirs exist, and the virus is not entirely eliminated. No therapy can presently be used to effectively remove integrated proviral DNA from the host genome[15,23–25].

To prevent viral rebounds, people living with HIV (PLHIV) must follow their treatment regimen strictly for the rest of their lives [26.] Additionally, even though ART-mediated viral suppression aids CD4 T cell recovery, this reversal represents an insufficient immune reconstitution, leaving PLHIV to deal with the issues of immunological failure for the rest of their lives[27,28]. These immunological failures could be as a result of changes in the DNA (nuclear and mitochondrial), accumulated over a long period of time, which leaves patients exposed to increased risk of metabolic disorders and oxidative stress[29].While ART-naive PLHIV has reduced mitochondrial activities, long-term ART exposure has been demonstrated to aggravate the negative effects that

were previously assumed to be exclusive to HIV infection[30]. NRTIs were the first group of ART medications to receive approval. NRTIs obstruct DNA polymerase gamma (Pol-), which is necessary for maintaining and replicating mitochondrial DNA (mtDNA), indicating ART as a potential contributor to mitochondrial malfunction[31]. Alternatively, despite the fact that NNRTIs, PIs, and INSTIs do not affect Pol- activity, they are also linked to mitochondrial dysfunction. Despite ART treatment lowering PLHIV mortality and reducing HIV-associated comorbidities, mitochondrial functions are nonetheless impaired even in clinically stable patients[32].

1.2 Statement of the Problem

Over the years, the Human immunodeficiency virus (HIV) has proven to be one of the life-threatening diseases in the world. This virus is a member of the Lentivirus genus, whose mode of action compromises the immune system, rendering the host susceptible to other diseases. Recent studies have shown HIV to cause severe damage to the host cell, including the human nuclear and mitochondrial DNA. These damages may result from DNA mismatch when proviral DNA has been integrated into the host genome and alterations occur during replication. ART also affects the nuclear and mitochondrial DNA in different ways by reducing copy number and increased oxidative stress and reducing the function of the DNA.

HIV-positive children born to HIV-positive women have been exposed to HIV and antiretroviral therapy in utero (from the womb). They are now on antiretroviral therapy for their own disease. The International AIDS Society classifies the short and long-term adverse genotoxic effects of antiretroviral treatment in HIV-positive adolescents under prolonged therapy as a key knowledge gap. This study hopes to bridge this knowledge gap in HIV-positive adolescents under antiretroviral therapy. The main focus of the study is the mitochondrial DNA (copy number and quality) in HIV-positive adolescents compared with HIV-negative adolescents in southwest

Nigeria. Reduced mitochondrial copy number and quantity can cause mitochondrial dysfunction which in turn leads to diseases such as cancer, diabetes, muscular dystrophy to mention few. Due to insufficient information about the possible genotoxic effects of HIV infection antiretroviral treatment and diseases resulting from prolonged treatment. we hope to find the level of damage caused to the mitochondrial DNA.

1.3 Justification of the Study

This study determined mitochondrial DNA damage (copy number and quality) in HIV-positive and HIV-negative adolescents in the southwest region of Nigeria. Hopefully, it demonstrated the adverse genotoxic effect of antiretroviral treatment in these patients and bridged the knowledge gap on the short and long-term effects of antiretroviral therapy in children and adolescents. This population has been exposed to ART either from birth or for a long period of time and when HIV enters the host genome as viral RNA, which is converted to proviral DNA, this integration could cause genomic instability as there could be errors during replication, thereby causing mutations in both nuclear and mitochondrial genome. Different ART regimens have various modes of operation and from previous research, these treatment affects the mitochondrial in different ways either by reduced copy number or by increased oxidative stress. As a result of mitochondrial dysfunction brought on by HIV and/or antiretroviral treatment, neurological dysfunction, malignancy, lipotoxicity, hyperlactatemia, and polyneuritis can all develop, because of this substantial adverse effect of ART, several elements play a part in their occurrence, including mitochondrial malfunction. Therefore, it is crucial to study mitochondrial dysfunction to understand the negative implications adequately.

1.4 Aim and Objectives of the Study

This study will focus on possible mitochondrial damage in HIV-positive adolescents on antiretroviral therapy from Lagos and Ibadan in southwest Nigeria. This study has two main objectives:

i. To determine and compare mitochondrial DNA copy numbers among HIV-positive and HIV-negative adolescents.
ii. To investigate mitochondrial DNA damage among HIV-positive adolescents compared to HIV-negative adolescents.

1.5 Research Questions

This study is centered on the following questions:

1. Will using antiretroviral drugs reduce mitochondrial DNA copy numbers among HIV-positive adolescents?
2. Do antiretroviral drugs administered to HIV-positive adolescents increase genome instability in mitochondrial DNA?

1.6 Hypothesis

Null Hypothesis: -There would be no significant difference in mitochondrial DNA damage levels among HIV-positive and HIV-negative adolescents.

Alternative Hypothesis: - The HIV infection and/or the use of antiretroviral treatment among HIV-positive adolescents would increase the occurrence of mitochondrial DNA damage compared with HIV-negative adolescents, i.e., significant difference would be observed.

1.7 Significance of the Study

Findings from this study will help to elucidate antiretroviral regimens with little or no genotoxic impact to be recommended for these adolescents who may have to be on antiretroviral treatment for life. It is expedient to ensure their offspring suffer no genotoxic ill effects. This pilot study will supply information to cover knowledge gaps on the long-term consequences of ART in HIV-positive adolescents. The findings would also be made available to other researchers who are in need of the information this study offers.

1.8 Scope of the Study

This study is set to determine the possible long-term genotoxic effect of antiretroviral therapy in HIV-positive adolescents undergoing treatment, with significant emphasis on the mitochondrial DNA damage (copy number and quality). The total number of samples to be used in this pilot study is 60 whole blood samples, 30 HIV-positive teenage patients undergoing antiretroviral treatment, and 30 HIV-negative patients from southwest Nigeria, specifically Oyo state and Lagos state. This research will be carried out for a period of six months. The mode of action of HIV and highly active antiretroviral treatment regimen will be considered. The mitochondrial DNA, damage, the effect of this damage, and potential diseases, as a result, will also be discussed.

1.9 Limitation of the Study

The major limitation of this study is being a pilot study, is the small sample size. However, we expect to increase the sample size in our subsequent investigation, depending on the study findings and direction the data will lead to further studies.

1.10 Operational Definition of Terms

I. **Mitochondrion**: This is referred to as the cell's powerhouse because it generates most of the chemical energy needed to power its biochemical reactions. The chemical energy produced in the mitochondrial is stored in small molecules called Adenosine triphosphate (ATP). It is also a membrane-bound cell organelle which contains its own chromosomes, and thus mitochondrial DNA is found in the mitochondrial[20].

II. **Mitochondrial DNA**: This is a small, circular chromosome within the mitochondria. It is different from the genomic DNA because it is smaller in size as it contains approximately 16,500 base pairs and encodes other proteins specific to the mitochondrial. It is specifically passed down from the mother to the offspring, unlike genomic DNA. When damage or mutation occurs in mitochondrial DNA, it causes mitochondrial diseases as insufficient energy could be produced[1,9].

III. **Mitochondrial Diseases**: This occurs as a result of mutations in the Mitochondrial DNA, resulting in insufficient energy production by the cells of various organs, thereby reducing the function of the organs due to cell malfunction. These errors occur during replication and exposure to various biological factors. Examples of mitochondrial diseases are Leigh syndrome, Pearson syndrome, and MELAS (Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes), to mention but a few. They are mostly inherited and therefore are passed down from parent to child and symptoms may start at birth or any age[21].

IV. **Human Immunodeficiency Virus (HIV)**: HIV is a virus that affects the immune system, specifically the CD4 count, thereby leaving the individual susceptible to other diseases such as cancer, tuberculosis, bacterial infections and many others and over time, it progresses to acquired immune deficiency syndrome (AIDS). It is a sexually transmitted infection through contact with of the genitals and exposure to infected sharp objects, for example, needles. There is no specific cure for HIV, but the illness is managed using antiretroviral therapy[12]

V. **Antiretroviral Therapy (ART)**: This is the combination of two or more drugs to inhibit the viral replication of HIV. ART individual drugs, operate at different stages of the HIV lifecycle, hence the different types and modes of operation. The use of multiple drugs acting on other targets is also referred to as highly active antiretroviral therapy (HAART), it inhibits HIV replication and reduces the HIV viral load in a patient. [22]

VI. **Mitochondrial DNA Copy Numbers (MtDNA-CN)**: This refers to the number of mitochondrial genomes per cell. It is a small invasive proxy test for mitochondrial activity and has been linked to various disorders that are age-related. Real-time PCR (qPCR) is the standard method for measuring mtDNA-CN, but it can also be quantified from genotyping microarray probe intensities and DNA sequencing read counts.[23]

VII. **Mitochondrial DNA Deletions/Damage:** Mitochondrial DNA deletions are caused by single large-scale changes of nucleotides in the mitochondrial genome. It occurs either through inheritance from the mother(oocytes) or arises de novo. It is the reason for mitochondrial damage and diseases.

VIII. **Singleplex PCR**: This is a process in polymerase chain reaction (PCR) used to detect one specific target sequence of DNA or RNA. Also used to detect a specific virus or bacterial genome of interest.

IX. **Multiplex PCR**: Unlike singleplex, Multiplex is a process in polymerase chain reaction (PCR) used to detect two or more target sequences of DNA and RNA simultaneously in a single sample preparation and amplification reaction. Multiple primers and probes, real-time PCR are added to allow more targets and analytes to be detected in one reaction.

X. **Polymerase Chain Reaction (PCR)**: This is a technique used in the laboratory to amplify small sections of DNA or RNA and proteins to make millions of copies of a targeted section of the sequence. It involves three major processes, Denaturation, Annealing, and Extension.

Endnotes

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Chapter Two

Literature Review

2.1 HIV Subtype Diversity Worldwide

There were 37.7 million HIV-positive individuals in the globe as of 2021[1], with sub-Saharan Africa hosting the majority of these people. Two genetically distinct lentiviruses, HIV-1 and HIV-2, which were spread by several cross-species transmissions of simian immunodeficiency viruses from nonhuman primates to humans, are the main cause of the global epidemic known as AIDS. The unique HIV-1 groups M (Major), O (Outlier), N (non-M, non-O), and the most recent group P were created as a result of these diverse zoonotic viral transmissions[2]. It has been shown that HIV-1 first appeared in the area of Kinshasa in the modern Democratic Republic of the Congo in the 1920s, from which point it spread via a transportation network to other regions in sub-Saharan Africa, West Africa, Europe, and the rest of the globe[3].

Multiple genetically diverse viruses were distributed in a geographically specified way throughout this worldwide dissemination. A range of subtypes and inter-subtype recombinants are found in Africa, with West Central Africa reporting the largest diversity. For example, subtype B became widespread in practically all of Europe and the Americas. Since the beginning of the HIV epidemic, group M viruses have predominated the world, whereas group N, O, and P viruses have not spread as widely. There are nine subtypes of Group M viruses (A–D, F–H, J, K)[2]. Genetic differences across subtypes are typically between 25 and 35 percent, although genetic distances within a subtype can range from 15 to 20 percent[4]. Although viral introductions have been detected elsewhere in Europe (Portugal and France), India, and the United States of America, HIV-2 remains primarily limited to the western region of Africa[5]. HIV-2 is made up of at least nine groups (formerly known as subtypes; A to I), of which groups A and D are currently circulating[5]. It has also been shown to be less

contagious than HIV-1[6,7]. Only a few recombinants have been reported, and there is currently a lack of information on HIV-2 subtypes[5,8].

2.2 Circulating Recombinant Forms of HIV

Recombination is a crucial event for viral diversification that enables the virus to avoid the host immune system and antiretroviral therapy[9]. A Virion known as a recombinant contains genome pieces from two or more different parental strains[10]. A circulating recombinant form (CRF) is a mosaic genome made up of areas various subtypes that develop from the mixing of viral genomes of various subtypes in dual-infected individuals. These recombinants are identified as circulating strains in the HIV pandemic and are categorized as CRFs when they are transmitted and spread within a community[11]. The virus has to be completely sequenced after being isolated from at least two unrelated people[12]. If there are more than three kinds, the term "complex" is used[13]. The HIV sequence database at Los Alamos National Laboratory now has 98 CRFs that have been discovered and updated[14]. As they are found and reported, these are assigned sequential numbers. The occurrence of different subtypes in West Africa is unknown, but according to current statistics, the region accounts for 16% of the world's HIV-1 cases, with the dominant HIV-1 subtypes being A (21%), G (35%), CRF02 AG (28%), and other recombinants (14%; the majority of which is CRF06 cpx), with the remaining subtypes accounting for less than 1% each. According to the same data, Nigeria has by far the highest number of HIV-1 infections in the region, with subtypes A (29 percent) and G dominating the epidemic (54 percent)[30].

2.3 Unique Recombinant Forms of HIV

Unique recombinant forms (URFs) are composed of a variety of subtypes, but in contrast to CRFs, they were only ever taken from one patient who had many infections[13]. As a result, whereas each URF exhibits distinct breakpoints, all sequences belonging to a CRF have the same recombination break sites in the genome. These viruses are not referred to as URFs even

if intra-subtype recombination does take place but does not result in subtype recombination. Although there haven't been any recorded cases of recombinants between HIV-1 and HIV-2, dual infections with both viruses have regularly been reported in areas where both viruses are prevalent[15]. In Eastern Africa, where these three subtypes cocirculate, several inter-subtype recombinants of AD and AC have been identified. Brazilian and Argentinean populations, where subtypes B and F are both prevalent, exhibit BF inter-subtype recombinants[16-19]. In India, recombinant AC and the subtypes A and C coexist[20].

2.4 Prevalence of HIV: Global Distribution of HIV-1 By Region

2.4.1 Africa HIV-1 Subtype Prevalence

Africa, particularly West Central Africa, has the most genetic diversity of HIV-1 strains (A, C, D, G, some also exist as combination of two strains), while other regions of the continent also exhibit a variety of distinct virus strains[21]. Subtype C collectively predominates in Southern Africa, whilst subtype A is most prevalent in Eastern Africa, despite the fact that additional subtype D and C viruses also cocirculate[21-25]. In Kenya, there has been considerable inter-subtype recombination as well as a rise in subtype C and a reduction in subtype D[24]. Based on almost full-length HIV sequences, recent research in Uganda found an increased incidence of HIV-1 inter-subtype recombinants of roughly 46%[26]. Based on deep sequencing of almost full-length viral genomes, different research by the Phylogenetic and Networks for Generalized HIV Epidemics in Africa (PANGEA)-HIV consortium revealed that Uganda had a significant percentage (approximately 50%) of HIV-1 inter-subtype recombinants (unpublished data)[27]. CRF02 AG viruses predominate in West and West Central Africa[21,28]. There have been reports of mosaic viruses involving CFR02 AG in numerous African nations and a rise in subtype F2 and other recombinant forms in Cameroon[29,30]. (Figure 2.1). Some nations, like Nigeria, have cocirculation of subtypes A and G[21]. In 1994, partial sequencing of four HIV-1 isolates in Nigeria revealed the presence of subtype G viruses. In the same year, a new HIV-1 strain (HIV-1 IbNg) was isolated in Ibadan, Nigeria. By 1996, a complete genome

sequence of HIV-1 IbNg had been obtained, and analysis revealed that IbNg is a complex mosaic genome with segments from subtypes A and G, giving rise to the designation CRF02 AG, of which IbNg is the prototype[30]. Recent research has revealed that subtypes G and CRF02 AG predominate in Nigeria. HIV-1 subtypes A, B, C, D, F2, G, J, and O have all been identified in Nigeria, with varying proportions of recombinant forms[30].

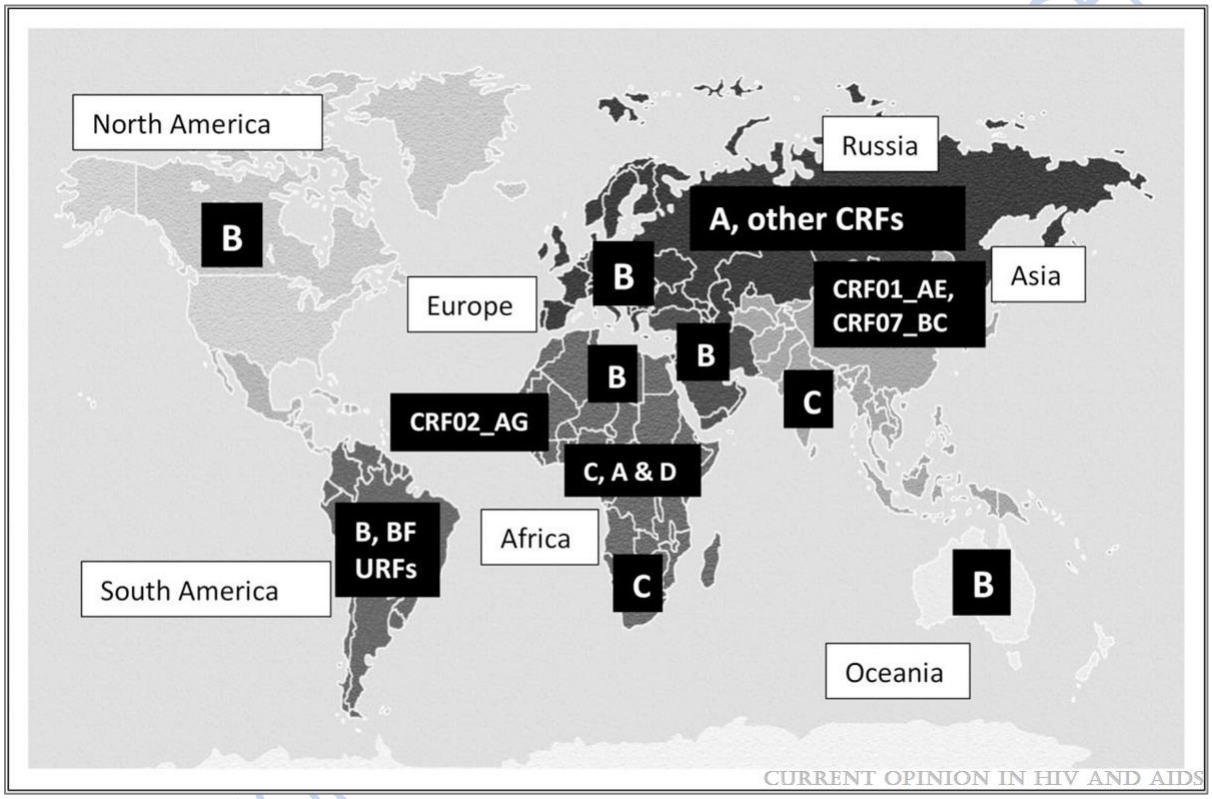

Figure 2.1: Global distribution of major HIV subtypes. Map showing the global distribution of the major HIV subtypes and circulating recombinant forms from the review. **Source** [29]

2.4.2 Europe and North America HIV-1 Subtype Prevalence

Subtype B is still the most common virus strain in North America, Europe, and Australia[31-37]. There have also been added new subtypes. For instance, subtype F1 and CRF02 AG were found in a cohort of MSM in Spain respectively[38,39].

2.4.3 Asia HIV-1 Subtype Prevalence

Asia has been referred to be the "hotbed" of recombinant viruses with several CRFs and is home to a number of subtypes[40-44]. Although CRF02 AG is widespread in Kyrgyzstan, Subtype A predominates in Russia and other former Soviet Union nations[45]. In China, CRF01 AE and CRF07 BC prevail, and more novel CRFs have been discovered there than anywhere else[46-52]. While subtype C predominates in India and there have been indications of a surge in URFs in the north-eastern region of the nation, CRF01-AE is the most common in southeast Asia[53,54].

2.4.4 Middle East and North Africa HIV-1 Subtype Prevalence

Countries in the Middle East and North Africa are grouped together because subtype B predominates in the area and these nations have relatively low infection prevalence and sampling rates[55-57].

2.5 The HIV Structure

HIV-1 Virion Structure is spherical and has a diameter of 100–130 nm (or roughly 1/10,000 mm). The viral envelope comprises about 7–12 trimeric complexes of viral envelope (Env) protein and A lipid membrane produced by the host cells composed of cellular proteins[58].The 72 knobs that make up the envelope are made up of the Env proteins trimers. The trimers of the gp120 (TM) surface protein are membrane-anchored by the transmembrane protein gp41(SU) as seen in Figure2.2[59].

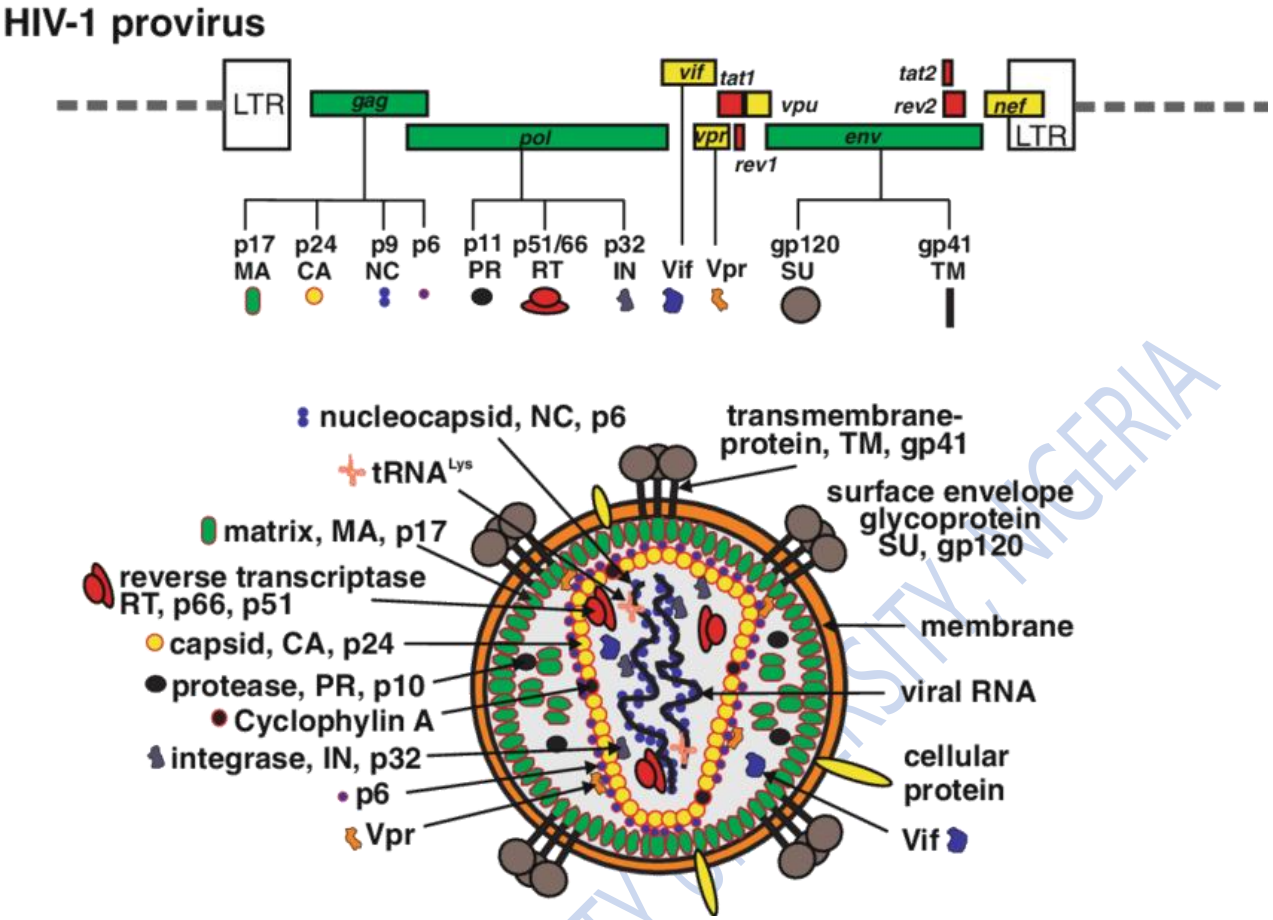

Figure 2.2: Schematic presentation of the expression of viral proteins that are found in the viral particle (upper) and of the mature HIV virion (lower). **Source**[58,59.]

The genome of the HIV virus consists of two identical single-stranded RNA molecules, which are enclosed in the core of the virus particle. Through the process of reverse transcription, the viral RNA is changed into double-stranded DNA known as proviral DNA. Long terminal repeats (LTR) sequence holds the degraded viral RNA side-by-side inside the proviral DNA that is incorporated into the human genome. HIV-1 genome consists of 9200-9600 nucleotides. The 5'end of the LTR region, which makes up the majority of the HIV-1 genome's 9200–9600 nucleotides, contains the promoters necessary for the viral gene's transcription[60]. The reading of the gap gene comes after the transcription of the proteins that make up the outer membrane (MA, p17), capsid protein (CA, p24), nucleocapsid (NC, p7), and a smaller protein that stabilizes nucleic acids. These transcriptions occur in the viral genome's 5' to 3' direction. Reverse transcriptase (RT, p51), protease (PR, p12), RNase H (p15), or RT plus RNase H combined with p66, and integrase follow the gap reading (IN, p32). Following the pol gene is the env reading frame, from which two envelope glycoproteins, gp120 (surface protein, SU) and gp41 (transmembrane protein, TM), are generated[59].

Table 2.1: Overview of HIV-1 proteins and their functions. **Source**[59]

Gene	Size	Protein	Function
Gag	P24	Prr55gag	Start of the inner structural proteins
	P17	capsid protein (CA)	Formation of the conical capsid.
	P7	matrix protein (MA)	Myristilated protein forms the inner membrane layer.
	P6	nucleoprotein (NC)	formation of the nucleoprotein/RNA complex, it is also involved in virus particle release
Pol	P10	Pr160GagPol	Precursor of viral enzymes
		Protease(pr)	Release of viral proteins and enzymes
	P51	Reverse transcriptase (RT)	Transcription of viral RNA into proviral DNA

	P15	RNaseH	Degradation of viral RNA in the viral RNA/DNA replication complex
	P32	Integrase (IN)	Integration of proviral DNA into the host genome.
Env	Gp160	Pr	Serves as a precursor for envelope proteins SU and TM. Held together by cellular protease.
	Gp120	Surface glycoprotein (SU)	It helps in attaching the virus to the target cell.
	Gp41	Transmembrane protein (TM)	It helps in anchoring gp120 during fusion to the viral and cellular membrane.
Tat	P14	Trans-activator protein	it activates the transcription of viral genes.
Vif	P23	Viral infectivity protein	It is necessary for infectious virus production in vivo.
Rev	P19	RNA splicing regulator	It helps in regulating the transport of partially spliced and non-spliced viral mRNA.
Vpr	P15	Virus protein r	Interacts with p6, it encourages the spread of the virus.
Nef	P27	Negative regulating factor	It promotes HIV replication. It increases the effect of viral particles.
Vpu	P16	Unique virus protein	Controls CD4 destruction ensures release of enough viral particles and controls its intracellular movement.
Vpx	P15	Virus protein x	Involved in the replication of the HIV-2 virus, it interacts with p6 in viral particles.
Tev	P26	Tat/rev protein	It regulates the activities of Tat and Rev in the nucleus, and it also controls the fusion of tat-env-rev.

Numbers correspond with the size of protein (p) and glycoproteins (gp) in 1000 Da.

2.6 HIV Mode of Entry into its Host Genome

HIV is one of the world's most deadly infectious diseases, especially in Sub-Saharan Africa, where it has considerably influenced health outcomes and life expectancy in recent decades. Two single-stranded RNA molecules are trapped within the center of the viral particle, which makes it an enveloped virus. The reverse transcription of the RNA of the virus into DNA, also known as proviral transcription, creates the genome of the HIV provirus, also known as proviral DNA. Reverse transcriptase, which is also encoded in the viral core, helps the viral RNA turn into DNA when it enters the host cell.[61]. Utilizing integrase and other components, viral DNA enters the cell nucleus and combines with nuclear DNA there. To prevent the immune system from detecting it, viral DNA remains dormant [62,63]. Replication of DNA takes place, increasing the amount of nuclear DNA and proviral DNA produced and released into the cell. HIV fuses with the CD4 on the T-helper cell through the aforementioned process, attaches to it, and transmits genetic material. As a result of the immune system's lymphocytes being destroyed, other highly infectious diseases can now attack the host[64].

The process to being infected with HIV involves several steps, starting with binding and fusion, during which the virus attaches to the host cell, followed by reverse transcription and integration, during which proviral DNA is created from RNA and integrated into the nuclear DNA of the host. The Gag and Gag/Pol precursor proteins (code p55 and p160) are cleaved into separate proteins of the mature HIV particle at the conclusion of the budding process and during the release of virions from the cell. In studies using electron microscopy, surface projection loss (SU trimers) caused by shear pressures, commonly known as shedding, can be seen following the release and then replication, where more copies of the proviral DNA are made in the cell and the final stage where the newly made viral DNA is released into the cell and set to infect other cells[65].

The human immunodeficiency virus is transmitted through contact with infected body fluids containing the virus. It can appear in blood, semen, vaginal fluid and breast milk. Although it might be present in urine and saliva, transmission through these is sporadic. HIV cannot be transmitted through hugs, kisses, handshakes, or mosquito bites, only through sexual activities, blood transfusion, pregnancy, and childbirth[14].

2.6.1 Stages of HIV Infection

HIV exists in different stages. The acute stage, where flu-like symptoms or no symptoms are noticed upon infection, is the most dangerous because it has the highest viral load. The second stage is chronic HIV infection. This infection is asymptomatic and clinical symptoms are not noticed until tested. Viral load is on the high side as CD4 counts decreases. The final and highest stage where the immune system is poor, CD4 is less than and 200per cubic milliliter and high susceptibility to other infections is called acquired immunodeficiency syndrome (AIDS) [65].

Kaposi's sarcoma and invasive cervical cancer are linked to a high prevalence of malignant tumors, including those that define AIDS (acquired immunodeficiency syndrome). In addition to these AIDS-defining malignancies, HIV-infected people are more likely to develop a number of cancers that do not necessarily indicate AIDS. The conclusion drawn from these observations is that HIV-1 can produce neoplasms prior to the start of AIDS. Recent studies suggest that HIV-1 infection results in reactions to chromosomal DNA damage. The specific chemical mechanism and biological relevance are still unclear, though[9]. A 15-kDa virion-associated nuclear protein is produced by the accessory gene VPR of the human immunodeficiency virus type 1 (HIV-1)[66,67]. It is a conserved gene that is necessary for the productive infection of macrophages and is present in both HIV-2 and the simian immunodeficiency virus[68,69].

2.7Antiretroviral Therapy (ART) and Types

Several studies have recently revealed that HIV-1 Vpr causes cell cycle abnormalities, resulting in cell aggregation in the G2/M phase and increased ploidy, resulting in DNA damage[14]. Various therapies, including the use of HAART (Highly active antiretroviral therapy), have been used over time to combat the fatal infection. After receiving an HIV diagnosis, a patient who tests positive will be placed on antiretroviral treatment, which involves giving them various medications. These medications are available in several types, each of which has a unique mechanism of action: Nucleoside Reverse Transcriptase, Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), HIV Integrase Strand Transfer Inhibitors (INSTIs), CCR5 antagonists, Protease Inhibitors, and Fusion Inhibitors, to name a few[70].

2.7.1 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

NRTIs were the first class of drugs discovered and approved by Food and Drug Administration (FDA). These drugs are administered as prodrugs, and it requires entrance into the host cells and phosphorylation by cellular kinases before they can act on the virus[71]. NRTI lacks a 3' hydroxyl group at the sugar moiety, which prevents the formation of a 3'-5' bond between NRTIs and the newly formed 5'nucleoside triphosphate, which results in the termination of the replication of the viral DNA. Chain termination can occur either at the DNA-dependent DNA or RNA-dependent DNA synthesis, thereby terminating the production of either the (+) or (-) strands of the proviral DNA in HIV[24]. There are now 8 NRTIs that have received FDA approval, including Abacavir (ABC, Ziagen), Didanosine (ddI, Videx), Emtricitabine (FTC, Emtriva), Lamivudine (3TC, Epivir), Stavudine (d4T, Zerit), Zalcitabine (ddC, Hivid), Zidovudine (AZT, Retrovir), and Tenofovir disoprovilfum, a nucleotide RT inhibitor[72].

Over time, treatment with these drugs will result in HIV strains that are resistant, i.e., with less drug susceptibility. Resistance to NRTIs occurs in two ways[73].

- ATP-dependent pyrophosphorolysis where the NTRIs are removed from the 3'end of the nascent chain, which reverses chain termination and discrimination between the native deoxyribonucleotide substrates and the inhibitors[74].
- NRTI integration into the development chain is prevented. The M184V/I and K65R mutations are linked to this pathway. Therapy with 3TC or FTC causes the M184V mutation to appear, but therapy with Tenofovir, ddC, ddI, d4T, and ABC can select the K65R mutation[74].

Many NTRIs mutations have shown the ability to reduce reverse transcriptase function and viral replicative fitness, and several studies have proven its clinical benefits, but it is important to note that even in the process of treatment, new mutations can occur resulting in a higher level of resistance[71].

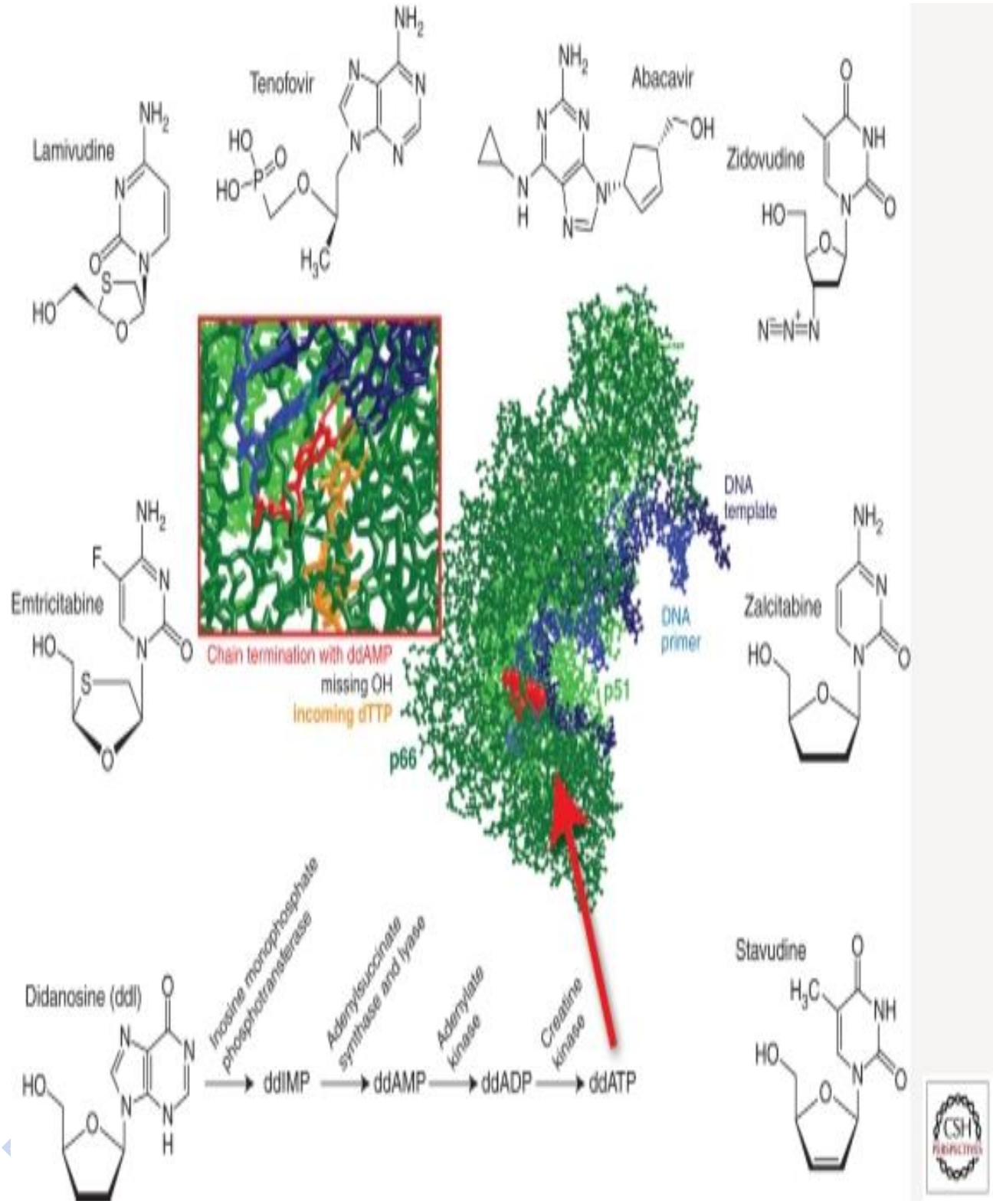

Figure 2.3**:** Nucleoside reverse transcriptase inhibitors and X-ray crystal structure of HIV-1 *RT in complex with DNA primer/template chain terminated with ddAMP and with an incoming dTTP. The cartoon of the crystal structure data was adapted from coordinates deposited by* Huang et al. (1998) *(1RTD).* ***Source***[28].

2.7.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Non-nucleoside triphosphate reverse transcriptase (NNTRIs) inhibitors are similar to the NTRI, but the mode of action is slightly different because they act on other sites of the enzyme. NNRTIs bind to HIV-1 RT and cause the creation of a hydrophobic pocket close to, but not enclosing, the active site[75]. This hydrophobic pocket inhibits HIV-1 RT and reduces polymerase activity by changing the spatial shape of the substrate-binding site. Hydrophobic residues (Y181, Y188, F227, W229, and Y232) and hydrophilic residues (K101, K103, S105, D192, and E224 of the p66 subunit and E138 of the p51 subunit) make up the NNRTI-binding pocket, which is present only when NNRTIs are present. These non-/uncompetitive inhibitors, in contrast to NRTIs, do not block the reverse transcriptase (RT) of other lentiviruses, such as HIV-2 and simian immunodeficiency virus (SIV)[76]. Currently, etravirine, delavirdine, efavirenz, nevirapine, and rilpivirine, which are in phase 3, are the four NNRTIs that are authorized[72].

Resistance of NNTRI is due to the substitution of amino acids in the binding pocket of NNTRI, such as L100, K101, K103, E138, V179, Y181 and Y188[77]. Similar to NRTI resistance, NNRTI resistance can manifest in diverse patterns, and alternate routes have been seen in non-subtype B afflicted people. The majority of NNRTI mutations result in some degree of cross-resistance between various NNRTIs, particularly when combined with additional secondary mutations[78]. Single nucleotide alterations in the case of NNRTIs can result in high-level resistance with just a minor loss of replicative fitness, as opposed to the large reductions in replicative fitness seen with resistance to other drug classes. NNRTI-resistant is more likely to spread and be stable due to a reduced genetic barrier, no effect on replicative fitness, and the gradual reversion of these mutations in patients in the absence of medication. Intriguingly, HIV-1 group O and HIV-2 frequently contain the wild-type sequence for the bulk of NNRTI-resistant mutations chosen during NNRTI treatment[78].

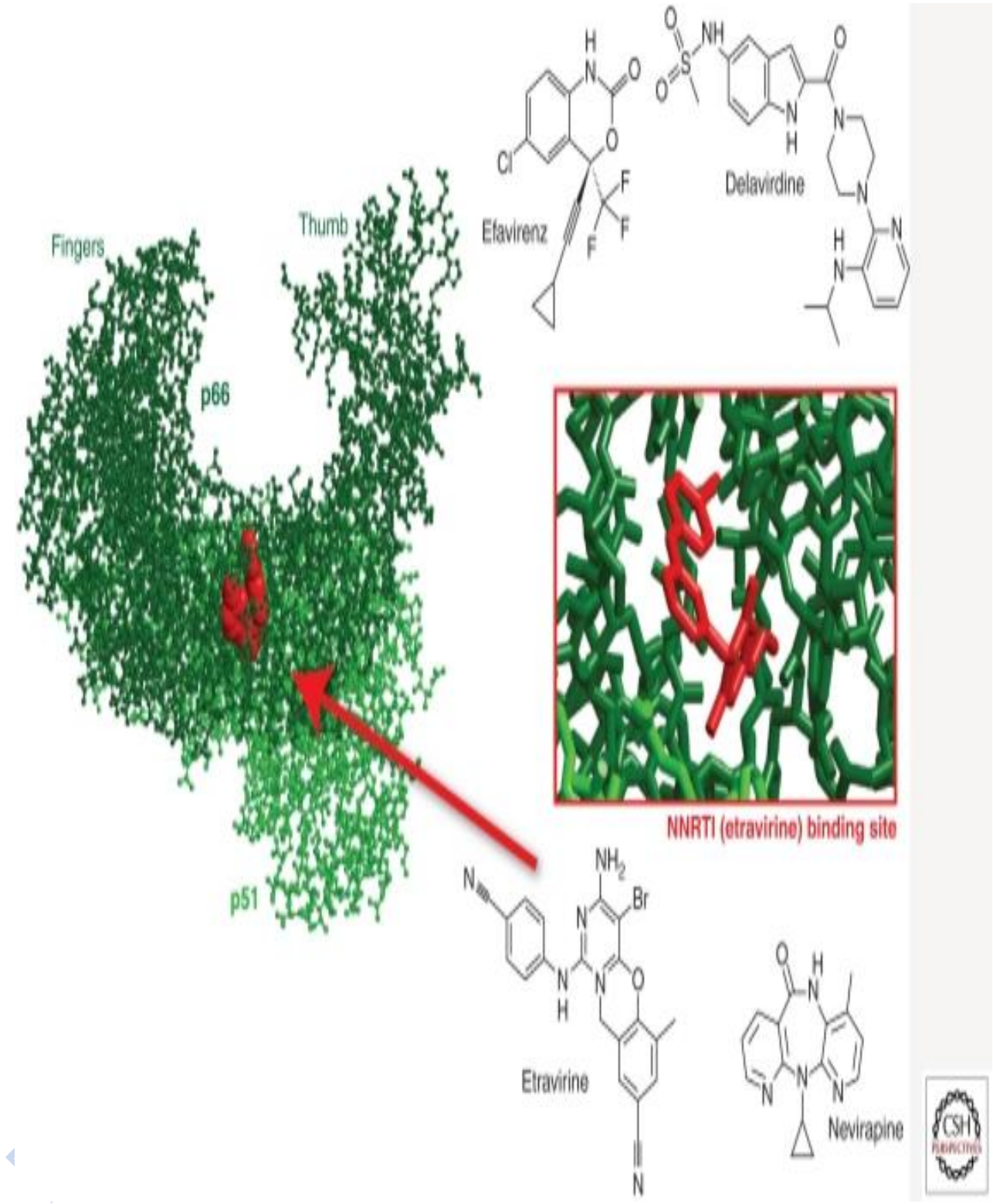

Figure 2.4: Non–nucleoside RT inhibitors and the X-ray crystal structure of HIV-1 RT complexed with etravirine. **Source**[75]

2.7.3 Integrase Strand Transfer Inhibitors (INSTIs)

HIV Integrase strand transfer inhibitors (INSTIs), research has shown HIV uses a protein called integrase to send its genetic material into its target cells. The enzyme integrase is the most recent HIV-1 enzyme successfully targeted for drug development. It facilitates viral DNA and strand transmission as well as 3′ end processing. Integrase inhibitors block this action, making it more difficult to develop drugs to treat the disease. INIs, or more specifically, integrase strand transfer inhibitors, are the terms used to refer to all integrase inhibitors now under research because they all work by inhibiting the strand transfer reaction (INSTIs)[79]. The primary mechanism of action involves interaction with the two essential magnesium metal ions cofactors in the integrase active site and the DNA, which causes cleavage to the particular complex between integrase and the viral DNA. A hydrophobic group that interacts with the viral DNA, the enzyme in the complex, and a metal-binding pharmacophore that isolates the active site magnesium make up all INSTIs[80]. The INSTIs are the only ART class interacting with two crucial parts of the virus: the integrase enzyme and the viral DNA. Resistance to this treatment class occurs due to specific mutations in the enzyme[69].

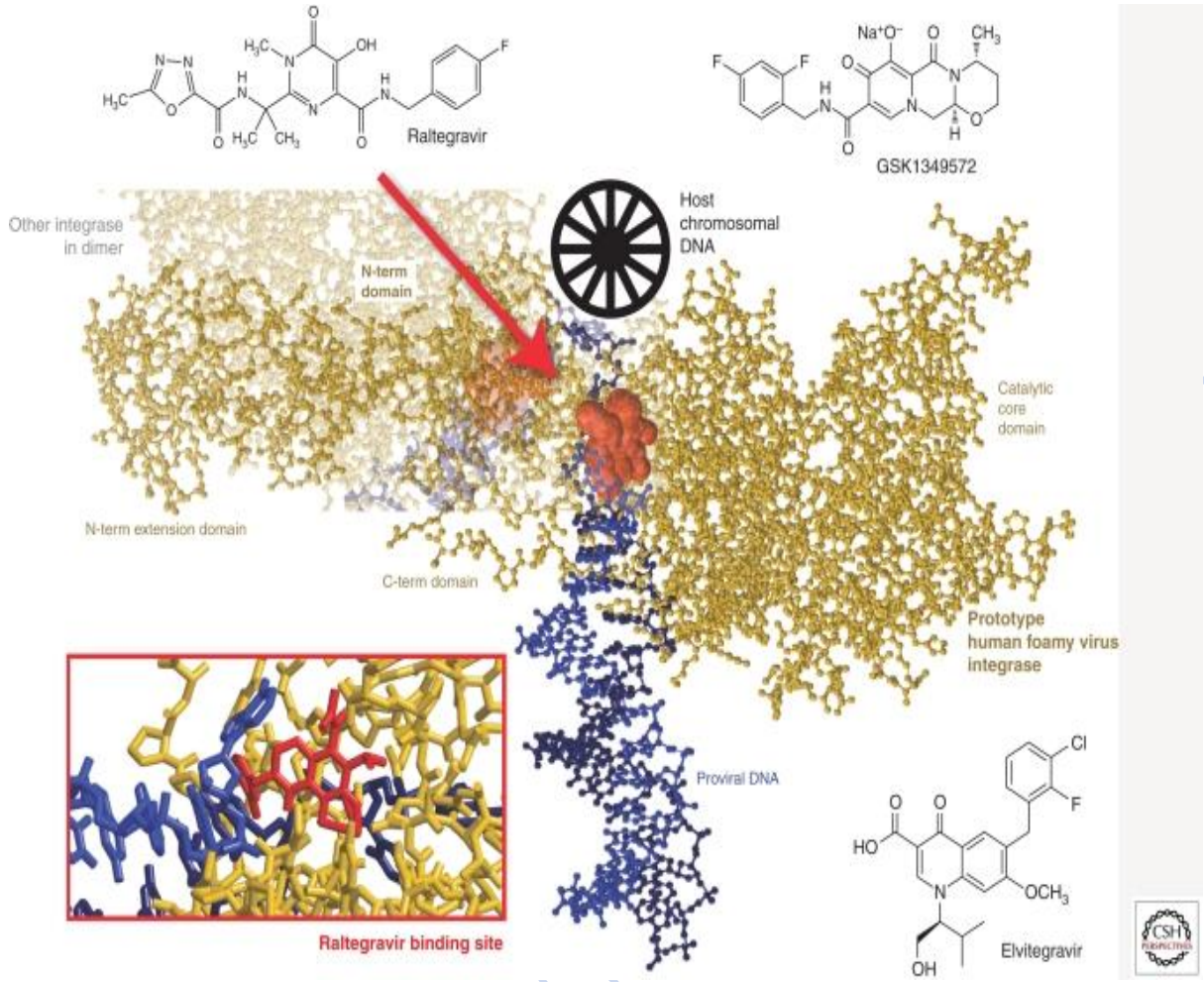

Figure 2.5: Integrase strand transfer inhibitors and the crystal structure of prototype human foamy virus integrase (as a model of HIV-1 IN) complexed to dsDNA and Raltegravir (3OYH). *N-term, amino-terminal; C-term, carboxy-terminal.* **Source**[75]

These different drugs attack the HIV operability mechanism in different ways, thereby increasing CD4 count and reducing the viral load of the host. When drug resistance rises, patients are placed under highly active antiretroviral therapy (HAART), a combination of different classes of ART, thereby reducing the level of resistance and serving not as a permanent cure for HIV but as an aid to survival against this deadly virus. It transforms HIV disease from a highly lethal pandemic to a chronic condition that requires meticulous management. HAART, on the other hand, does not attack the integrated proviral DNA. As a result, antiviral medications must be used for a long time to maintain life[77].

As a result of mitochondrial dysfunction brought on by HIV and antiretroviral treatment, neurological dysfunction, malignancy, lipotoxicity, hyperlactatemia, and polyneuritis can all develop. Because of these substantial unfavourable repercussions, several elements play a part in their occurrence. A common factor was found to be mitochondrial malfunction. Therefore, it is crucial to study mitochondrial dysfunction to understand the negative implications adequately. I will look at the mitochondrial DNA damage in teens in southwest Nigeria who are HIV-positive in this study.

2.8 Mitochondrial DNA Structure, Function and Damage

Mitochondria are ubiquitous double-membrane subcellular organelles present in all nucleated mammalian cells. Their primary function is to support aerobic respiration and produce, by oxidative phosphorylation (OXPHOS), the bulk of cellular energy called Adenosine Triphosphate ATP[81]. This is possible due to phosphate, a high-energy bond that provides energy for other cell activities. The primary purpose of the mitochondria is to produce energy. Different cells contain different numbers of mitochondria because the energy consumed varies from cell to cell. In the mitochondria, the mitochondrial DNA can be found, which is one of the main focus of this study.

Human Mitochondrial DNA (mtDNA) is a 16,569 base pair circular chromosome, the double-stranded, supercoiled molecule that encodes for 37 contiguous genes that are required for OXPHOS and mitochondrial protein synthesis. It was initially found in 1963, and then sequenced in 1981 by Sanger[82]. Based on the 'Endosymbiotic theory,' mtDNA was derived from the circular genome of bacteria engulfed by the ancestors of the present-day Eukaryotic cells. It is different from nuclear DNA as it is smaller in size and was first sequenced, it lacks introns, and in the 37 genes encoded in it, 13 genes are involved in OXPHOS, and 22 genes encode for transfer RNAs for specific amino acids. Two genes encode for 2 Ribosomal RNA. The human mitochondrial DNA is made of two strands; heavy and light strands where these genes are located. The heavy strand contains 14 transfer RNAs, two ribosomal RNAs (12S and 16S), and 12 oxidative phosphorylation system subunits. It is also rich in guanine (tRNAs). Eight tRNAs and one subunit are encoded on the light strand[83,34].

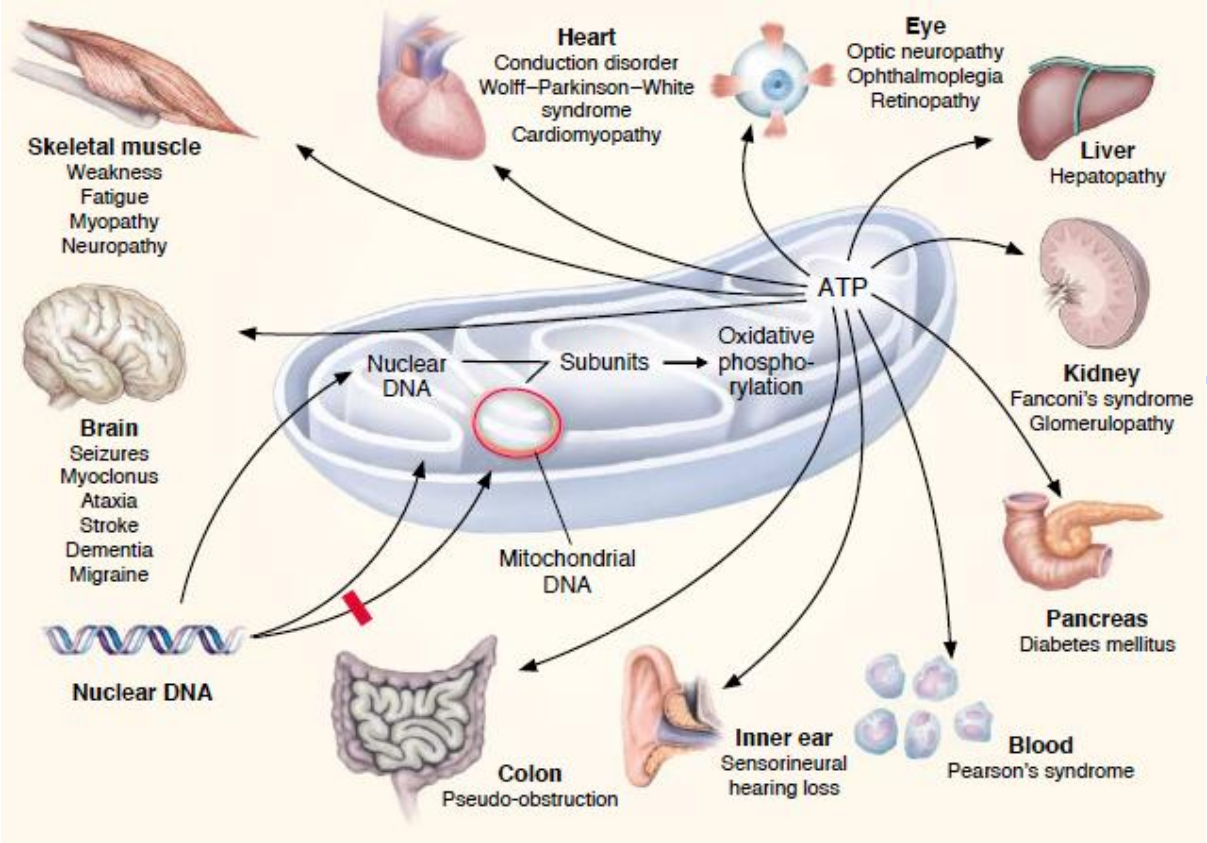

Figure 2.6: Interaction between Genes Encoded by Nuclear DNA and Those Encoded by Mitochondrial DNA in Oxidative Phosphorylation. **Source**[11]

The OXPHOS system is made up of five multi-subunit enzyme complexes that are located on the inner mitochondrial membrane. NADH-ubiquinone oxidoreductase or complex I, Succinate dehydrogenase or complex II ubiquinone-cytochrome c oxidoreductase or complex III, cytochrome c oxidase or Complex IV and cytochrome c oxidase ATP synthase or complex V. These complexes are needed for the proper function of the mitochondrial genome[85].

The mitochondrial genome from the sperm is destroyed during fertilization, leaving only the mtDNA in the oocytes to be passed down to the offspring. Even though mtDNA is passed down from mother to child over generations, it retains its variability due to high mutation rates caused by the lack of repair mechanisms and proofreading abilities. Ten times more mutations occur in mitochondrial DNA than in nuclear DNA, which may be due to a nucleotide imbalance in the mitochondria that lowers DNA polymerase gamma fidelity and increases the mutation rate of mitochondrial DNA[86]. By examining the brief variable sections found in the non-coding area of the mtDNA, it is possible to assess the evolutionary phylogeny and use this variability in human identity testing, migration and ancestry mapping, and evolutionary phylogenetic analysis.

MtDNA has recently been found to have a significant role in innate immune responses, and inflammatory pathology and can also function as a unique marker for the prognosis of many diseases, including HIV, cancer and some other diseases[87,88].

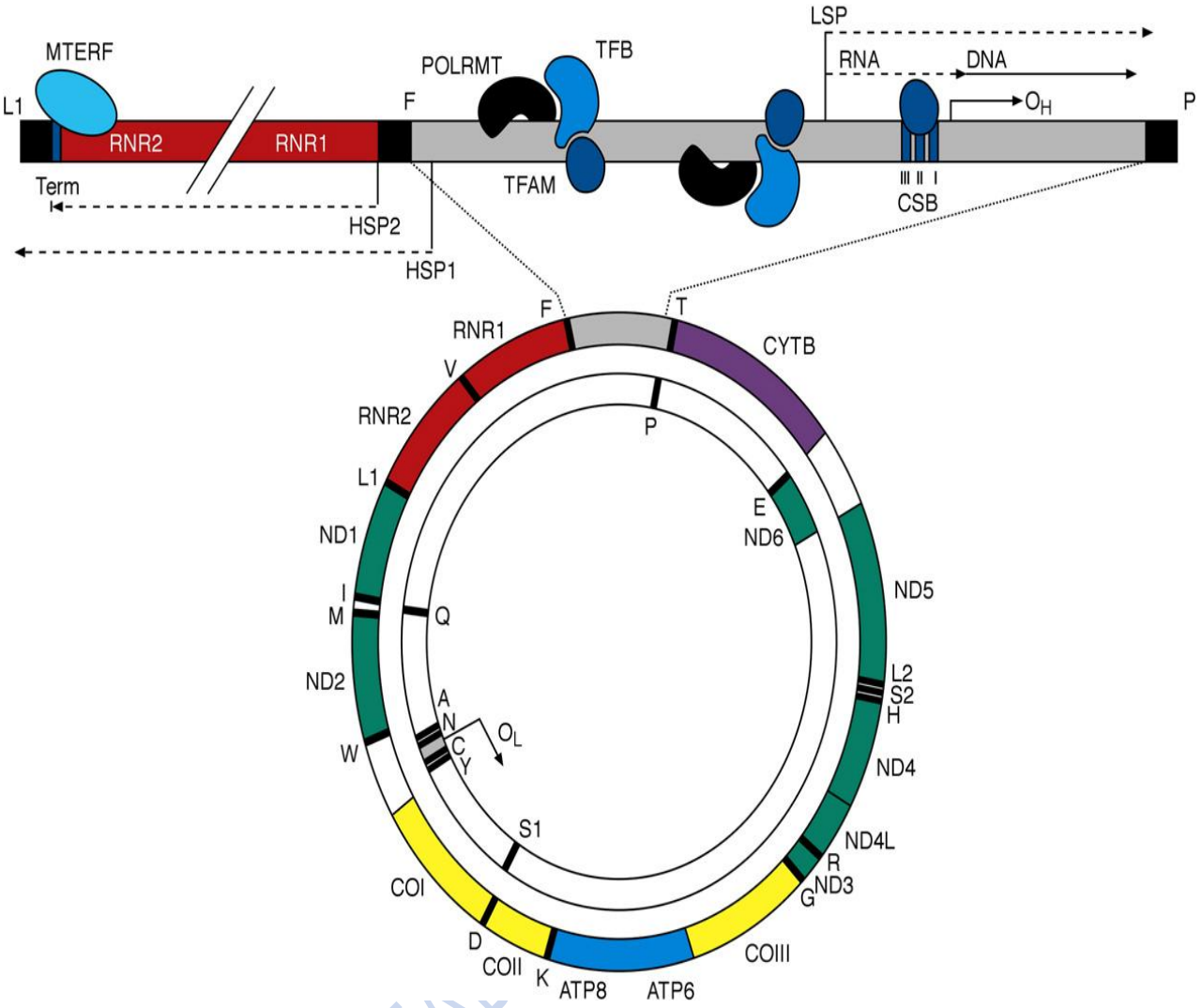

Figure 2.7: Human mitochondrial genome. **Source**[59].

Represented is a schematic diagram of the 16.6 kb circular, double-stranded human mitochondrial genome with an enhanced view of the mammalian D-loop and transcription termination regions, shown in linear form. The circle represents the heavy (H) strand of the genome and the inner circle the light (L) strand. Human mitochondrial DNA encodes the 2 mt-rRNAs (red) RNR1 (12S rRNA) and RNR2 (16S rRNA), 22 mt-tRNAs (black bars), identified by their single letter abbreviation, and 13 essential RC polypeptides: ND1-ND4 and ND4L, subunits of complex I (green), CYTB, a subunit of complex III (purple), COI-COIII, catalytic subunits of complex IV (yellow), and ATP6 and ATP8, subunits of complex V (blue). Major non-coding regions of the genome (grey) include the 1.1 kb D-loop, and the origin of L-strand replication (OL). The origin of H-strand replication is indicated within the D-loop (OH). H-strand transcription is initiated either from HSP1, generating a short transcript that terminates at the RNR2/MTTL1 boundary (Term) under the guidance of the transcription termination factor MTERF, or from HSP2, generating polycistronic transcripts of the entire H-strand. LSP denotes the L-strand initiation point that produces polycistronic transcripts for this strand and also generates RNA precursors for H strand replication initiation. Conserved sequence blocks (CSBs I-III) are conserved regions in human, mouse and rat that participate in the formation of RNA primers for replication. Transcription from all promoters requires the upstream binding of transcriptional activator TFAM, together with a single subunit RNA polymerase (POLRMT), which forms a heterodimeric complex with the transcription factor TFB2M (depicted as TFB). TFAM also binds to other regions of the D-loop; however, only binding to the CSB region is shown[59].

Some areas in the genome of the mitochondrial DNA are susceptible to deletions, and some are not. The majority of mtDNA deletions have been observed to occur in this significant region of the mitochondrial DNA, which is between the origin of the heavy strand replication [O_H (nucleotides 110-441)] and the origin of the light replication strand [O_L (nucleotides 5721-

5798)][89]. Hence, ND4 is often deleted in many patients, and ND1 outside the region is less susceptible to deletions[90]. The main focus of this study will be the copy number level of these genes in patients with a compromised immune system by HIV and HAART.

Numerous studies have demonstrated that HIV and ART can result in deletions in the mitochondrial DNA in adults, making the patient more vulnerable to various illnesses. However, little research has been conducted on teens, leading to the design of this pilot study.

2.9 The Effect of Mitochondrial on HIV Infection and ART Therapy

Given how many vital functions mitochondria play, any influence from outside factors, such as HIV infection or ART therapy, may be damaging to mitochondrial functioning. Numerous downstream consequences, many of which are essential for cellular survival and function, result from the dysregulation of mitochondrial activity.

Evaluation of mtDNA copy number, mtDNA mutations, ROS generation, mitochondrial membrane potential (ΔΨm), cellular respiration, apoptosis, and ATP production are some of the most important measurements of mitochondrial activities now available. The metabolic and genomic condition of cells after probable medication and HIV toxicity may be evaluated using these criteria. There is currently a gap in knowledge on how HIV and ART therapies interact to cause mitochondrial dysfunction, how these mechanisms may work together to cause mitochondrial dysfunction, and how these processes may be responsible for the immunological ageing seen in PLHIV on ART[91].

2.9.1 HIV-Induced Mitochondrial Dysfunction: The Influence of Virally Encoded Proteins

Different cell types have been shown to regulate mitochondrial activity differently as a result of HIV infection. For instance, research has demonstrated a negative association between m and the proportion of apoptotic cells and that mitochondrial membrane potential (ΔΨm) is decreased in HIV-infected, ART-nave patients compared to HIV-negative healthy people. In

HIV-infected, ART-unexperienced people, CD4+ T cell counts have been demonstrated to positively correlate with the change in $\Delta\Psi m$[91,92]. It is possible that some of the functions of proteins encoded by viruses contribute to mitochondrial dysregulation. Several proteins required for viral replication and integration are encoded by HIV-1, including the structural proteins Gag, Pol, and Env, as well as the regulatory proteins Nef, Vpr, Vif, and Vpu. According to published research, a number of these virally encoded proteins, such as Env, Vpr, Tat, Nef, and Vpu, contribute to the apoptosis that HIV causes in cells and is mediated by mitochondrial activity[93–99]. Each of these proteins has the ability to trigger a process known as bystander-induced apoptosis, in which infected cells that are producing virally encoded proteins interact with nearby uninfected cells to cause death. One of the most frequently accepted theories for how HIV infection results in the loss of CD4+ T cells at a pace that differs from viremia levels is the one described above[91,93-100]. Consequently, HIV has a major impact on mitochondrial homeostasis via deregulating the $\Delta\Psi m$ and cellular apoptosis.

2.9.2 HIV-Encoded Env: A Regulator of Viral Infection, Apoptosis, and Mitochondrial

The HIV-gene env produces the viral envelope-forming protein Env, which has been implicated in regulating cell death in several studies. The 160 kD glycoprotein that the env gene specifically genes for are broken into the noncovalently related proteins gp41 and gp120.

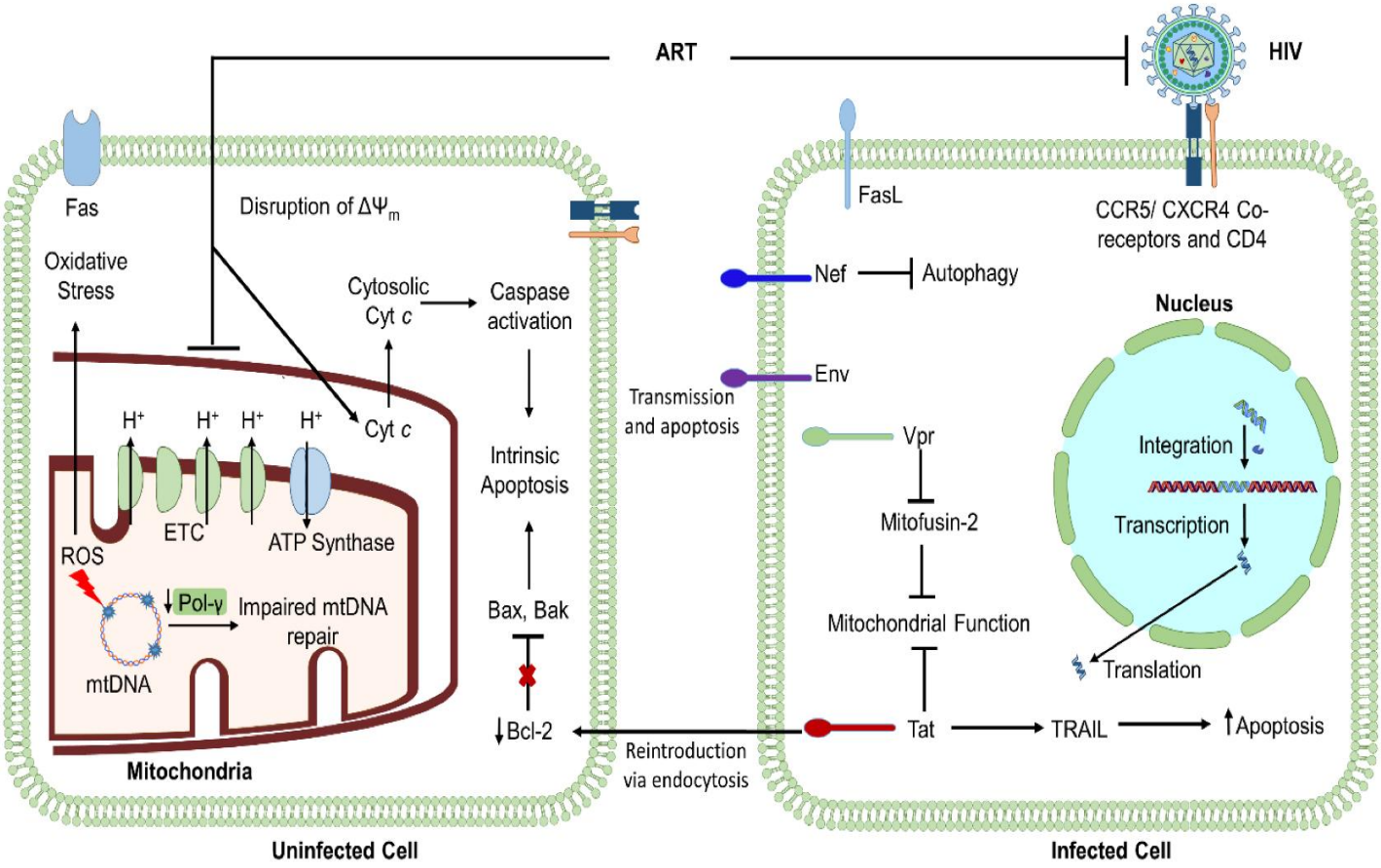

Figure 2.8: Model of cooperative induction of mitochondrial compromise by HIV-infection and ART-treatment. *Mitochondrial dysfunction is mediated by viral integration, disruption of mitochondrial membrane potential (ΔΨm), decreased respiration and ATP synthesis, mtDNA mutations, impaired mtDNA repair, intrinsic apoptosis, and oxidative stress. Collectively, these deregulations facilitate energetic failure and depletion of CD4 T cells.* **Source**[99].

By being outside of the viral membrane, the N-terminal component, gp120, enables brief interactions with target cells' CXCR4 and CCR5 coreceptors. Through abnormal calcium transport and signalling, mtDNA instability, and mitochondrial-facilitated apoptosis, this interaction not only facilitates entrance into cells to initiate infection but also frequently results in cell death[101]. Gp120 triggers apoptosis by depolarizing the mitochondrial membrane, which disrupts ATP generation, gluconeogenesis, and ETC function. Cytochrome c is then released, activating caspases 9 and 3, which triggers cellular death (Figure 2.8). Env is essential for interaction with uninfected neighbouring cells due to its localization in the cell membrane of an infected cell and interactions with nearby cell surface receptors (see Figure 2.8), which also helps to facilitate infection via interactions with coreceptors and the glycoproteins found on the virion surface[102,103]. Thus, the link between cells expressing virally encoded proteins and bystander-induced apoptosis may offer crucial insights into the process by which HIV infection results in a decrease in CD4+ T cell counts and early immunological aging[103].

2.9.3 HIV-Encoded Vpr: A Regulator of Apoptosis and Mitochondrial Function

Vpr can cause HIV-induced apoptosis in addition to the mitochondrial dysregulation caused by virally-encoded Env. A supporting protein called Vpr is crucial for the advancement of viral integration because it makes it easier for the pre-integration complex to enter the nucleus of the host cell, where it can then integrate the viral genome into the host DNA[104,105]. Vpr-mediated activation of the intrinsic death pathway via cytochrome c and caspase activation, similar to Env activity, is the basis for the capacity of the Vpr gene product to trigger cellular apoptosis[102,106]. Additionally, thorough mutation analysis of the HIV-1 ORFs showed that Vpr and Vif together have the power to cause cell death and G2 cell cycle arrest [107]. Studies have also shown that the mitochondrial fusion protein mitofusin-2 is damaged by Vpr, which compromises the mitochondria (Mfn2). Mfn2 is essential for controlling mitochondrial activities such as fusion, transport, turnover, and interaction with other cellular organelles.

Thus, Mfn2-mediated connections between the endoplasmic reticulum (ER) and mitochondria in human peripheral blood mononuclear cells (PBMCs) are altered by Vpr-induced damage to Mfn2 (Figure 2.8). The integration of the C-terminal transmembrane domain is probably how Vpr is able to localize to the ER, mitochondria-associated membranes (MAM), and mitochondrial outer membrane (MOM). The disruption of the MOM's structural stability caused by Vpr has also been linked to lower levels of Mfn2 protein expression[108].

2.9.4 HIV-Encoded Tat: A Regulator of Apoptosis and DNA Damage Repair

The Tat protein, which is known to be generated in infected cells and released and reabsorbed into neighbouring uninfected cells via endocytosis, is also encoded for by the HIV genome (Figure 2.8). Through CCR5 and CXCR4 receptors, Tat may also cause apoptosis, participate in the bystander effect, and infect nearby cells.

According to published research, Tat triggers apoptosis by controlling the traditional intrinsic apoptosis mechanism. To release the proapoptotic agent cytochrome c as well as the proapoptotic FasL and Bax, Tat can specifically downregulate the antiapoptotic protein Bcl-2 and upregulate the proapoptotic caspase-8[109]. Literature also demonstrates that Tat increases macrophage production of TNF-related apoptosis-inducing ligand (TRAIL) (Figure 2.8) and that T cells from PLHIV-positive individuals are more susceptible to TRAIL-induced apoptosis[110].

Additionally, it has been demonstrated that Tat mechanically inhibits the telomerase enzyme. Telomerase lengthens the telomeres, which are hexameric DNA repeats at the ends of chromosomes, to repair DNA shortening brought on by continuous replication. Tat exposure alone was able to cause this telomerase decrease, despite the fact that telomerase has been proven to be diminished in CD4 T cells infected with HIV. Tat can specifically inhibit telomerase reverse transcriptase (hTERT), the catalytic component of telomerase, from expressing in the nucleus and disrupt the AKT pathway, which is essential for hTERT

activation[111]. Tat has also been demonstrated to inhibit p53, a cell cycle regulator, from being expressed[112,113]. These findings point to a further mechanism by which Tat might impair the capacity for replication of both infected and uninfected CD4 T cells, potentially causing a reduction in the number of functional CD4 T cells in PLHIV. A possible mechanistic connection between telomeric DNA damage and mitochondrial dysfunction has also been discovered in several investigations via a p53-dependent route[114]. These results show Tat promotes mitochondrial dysfunction through a variety of different routes.

2.9.5 HIV-Encoded Nef: A Regulator of Apoptosis and Mitophagy

Nef, a protein that is encoded by a virus, is necessary for T cell activation and sustaining chronic infection[115]. Nef causes nearby T cells to become infected via the CCR5 and CXCR4 receptors, activating T cells without the need for the TCR, and aiding in the depletion of CD4+ T cells. According to published research, Nef-expressing T cells also produce FasL, which enables them to kill uninfected T cells that are Fas-expressing, demonstrating that Nef has multiple mechanisms for inducing bystander apoptosis. Through two proapoptotic structural features, Nef also causes apoptosis[112]. Nef has also been demonstrated to control the autophagic and mitophagic processes (the selective degradation of damaged mitochondria via autophagy). Nef most frequently suppresses autophagy by interacting with key proteins in autophagy start, such as Beclin-1[116], allowing for viral persistence. Continuous suppression of autophagy and mitophagy from Nef interactions may result in a disturbance of mitochondrial functioning and an accumulation of dysfunctional mitochondria because autophagy is essential for maintaining cellular homeostasis and mitochondria are the primary source of energy[117]. Consequently, ROS levels would rise and mitochondrial DNA damage would worsen.

2.9.6 HIV-Mediated Mitochondrial Compromise

Genes that control cell death, cell activation, and inflammatory chemokines and cytokines are enriched in PBMCs, muscle, and adipose tissues from people with latent HIV, whereas genes that control mitochondrial function and biogenesis are downregulated in both PBMCs and adipose tissues from people with long-term controlled HIV viral load[115,118]. As a result, infection also has a direct impact on inflammation, immune cell activation, and mitochondrial function, all of which are potential contributors to the early aging associated with HIV infection[119]. It is crucial to keep in mind that HIV-induced apoptosis via depolarization of the mitochondrial membrane also causes mtDNA mutations and disrupts ATP synthesis, OXPHOS, and ROS generation[120]. Disruption of the ΔΨm, which prevents the removal of dysfunctional mitochondria and the transfer of vital ions and proteins necessary for mitochondrial functioning, demonstrates how crucial the m is to preserving cell viability and homeostasis[116,121].

In addition, studies have shown that PLHIV are more likely to age prematurely, which is associated with higher oxidative stress and damage as well as short telomeres, which may further exacerbate the compromise of mitochondria and metabolism[121-125]. In a study examining the role of mitochondria in ART-naive and ART-exposed PLHIV, it was shown that the loss of CD4+ T cells resulted in increased mitochondrial mass in both CD4+ and CD8+ T cells. In CD8+ T cells, HIV also seemed to target ΔΨm, which increased the production of ROS in CD4+ T cells. Following ART therapy, both cell subsets had a marked reduction in mitochondrial mass, which was followed by a rise three years into the course of treatment. These findings show that CD4+ and CD8+ T cells differed significantly in their dynamic fluctuation of immune cell response to HIV infection and ART-mediated suppression of infection[126]. Deregulation of mitochondrial parameters and increased oxidative stress can set off a vicious loop that results in excessive ROS generation, mtDNA damage, mitochondrial failure, and apoptosis, further compromising metabolic health in HIV-infected individuals.

The latently HIV-1-infected T cell line ACH2 was shown to have much higher quantities of viral RNA localized in the mitochondria after TCR stimulation compared to the cytoplasm and nucleus, according to a study looking at the intracellular distribution of HIV-1 RNA in infected cells. In comparison to acutely infected cells, chronically infected cells have much less viral RNA localized in their mitochondria[127]. Furthermore, mitochondria from HIV-infected H9 cells can be transferred into uninfected cells (MT2 target cells) to aid in viral transmission, according to live-cell real-time fluorescence imaging. In addition, infection was produced when uninfected cells were cultured with isolated mitochondria from HIV-infected T cells. This infection was indicated by the production of the viral antigen p24, the development of syncytia, and the depletion of target cells. This infection was avoided by culture in the presence of pharmacological inhibitors of mitochondrial function[128]. Thus, HIV-infected cell mitochondria can serve as viral reservoirs to promote cell-to-cell infection. Additionally, research found that long-term non-progressors (LTNPs), or PLHIV, had considerably lower rates of apoptosis and mitochondrial impairment in PBMCs than untreated asymptomatic typical progressors (TPs), who have high viremia and immunological degradation[129]. These findings suggest that mitochondria may have a conditional impact on HIV infection, viral latency, and disease severity. In order to promote greater viral transmission and infectivity at varied levels during various phases of HIV infection, it is therefore plausible that HIV deliberately hijacks mitochondrial function.

Due to the crucial involvement of mitochondria in innate and antiviral immune responses, modulating mitochondrial activity may be the best strategy for the virus to promote infection[130]. HIV presumably targets mitochondrial function by mtDNA copy number reduction and dysregulation of m and mitochondrial signaling in order to prevent mitochondria-mediated HIV-clearance. The effects of HIV infection as a whole impact viral contagiousness and ultimately result in mitochondrial impairment. This suggests that there is a

well calibrated equilibrium between utilising mitochondria for transmission and for reducing immunological responses to HIV infection.

2.10 ART-Induced Mitochondrial Dysfunction

Even while mitochondrial functions are suppressed in ART-naive PLHIV, long-term ART exposure has been demonstrated to exacerbate side effects that were previously assumed to be exclusive to HIV infection[131-135]. In reality, in the era of ART, the effects of a persistent infection are less severe than those of a long-term ART regimen, particularly for individuals who are aviremic. Nucleoside-analog reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INIs), which include the subclass integrase strand transfer inhibitors (INSTIs), fusion inhibitors, and coreceptor antagonists are among the classes of ART that have been approved for the treatment of HIV. Each of these NRTIs were the first group of ART medications to receive approval. NRTIs obstruct DNA polymerase gamma (Pol-), which is necessary for maintaining and replicating mitochondrial DNA (mtDNA), indicating ART as a potential contributor to mitochondrial malfunction[102]. Alternately, despite the fact that NNRTIs, PIs, and INSTIs do not affect Pol- activity, mitochondrial dysfunction is also linked to this medication groups[136]. Despite the fact that ART therapy lowers the risk of PLHIV death and reduces comorbidities associated with HIV, mitochondrial functions are nevertheless impaired even in clinically stable individuals[137].

The administration of combined ART makes it more challenging to evaluate the disturbance of mitochondrial function caused by separate ART regimens. It may be possible to enhance treatments and quality of life for PLHIV by comprehending the special mechanisms through which HIV infection in conjunction with ART causes mitochondrial dysfunction. For the purposes of this review, we will concentrate on the mitochondrial toxicity linked to NRTIs, NNRTIs, PIs, and INSTIs because of the prevalence of their inclusion as well as the evidence

demonstrating that these medications have more adverse effects than fusion inhibitors and coreceptor agonists[138,139]. Table 2.2 provides an overview of these findings as well as mitochondrial dysregulation by all ART classes.

Table 2.2: Mitochondrial dysregulation by ART classes. **Source**[102]

Drug Class	Mechanism of Action	Mitochondrial Dysfunction	Species and Cell Type Models
NRTIs (Abacavir, Tenofovir)	Prevents viral replication by inhibiting HIV reverse transcriptase	Inhibition of Pol-γ Reduction of mtDNA copy number/mitochondrial encoded proteins Decreased proliferation of lymphocytes Lack of respiratory chain blocking ETC complexes ATP Decrease upsurge in oxidative stress, Reduction in Ψm	Human fibroblasts, PBMCs, CD4, and CD8 cells, and rat liver cells
NNRTIs (Rilpivirine, Efavirenz, Nevirapine)	Blocks viral replication throughco-operative binding to HIV reverse transcriptase	Respiratory chain shortage, ATP decrease Increased oxidative stress Decrease in Ψm Apoptosis	Human hepatic cells, PBMCs, coronary artery endothelial cells, and hepatoma cells, and Jurkat T cell line
PIs (Ritonavir, Darunavir, Atazanavir, Indinavir, Saquinavir)	Prevents viral replication by inhibiting HIV protease	Increased oxidative stress Human CD4, CD8, macrophage-derived foam cells, endothelial hepatoma, and hepatic cells, and Huh-7.5, 293T, HeLa, and Hepa RG cell lines Reduced mtDNA copy number Respiratory chain deficiency Reduced ATP Apoptosis	Human CD4, CD8, macrophage-derived foam cells, endothelial, hepatoma, and hepatic cells, and Huh-7.5, 293T, HeLa, and Hepa RG cell lines
INIs (Raltegravir,	Prevents integration of	Respiratory chain	Human CD4 and CD8

Dolutegravir, Elvitegravir)	viral DNA into the host genome by inhibiting HIV integrase enzyme	deficiency Increased oxidative stress Increased cytoplasmic mtDNA copy number	
Fusion Inhibitors (Leronlimab, Ibalizumab, Enfuvirtide)	Prevents viral fusion with target cell membrane by binding to the viral envelope protein gp41	Not identified	N/A
Coreceptor Antagonists (Aplaviroc, Maraviroc, Vicriviroc)	Prevent viral infection by interfering with viral entrance into the cell by blocking the coreceptors, such as CCR5 or CXCR4, on the surface of target immune cells	Not identified	N/A

2.11 Different ART-Induced Malfunction in The Mitochondrial

The functionality of mitochondrial can be impacted by many variables, including aging, infections, and some antiretroviral medications. These elements may harm the mitochondria, impairing the cell's ability to operate normally. Mitochondrial toxicity, a generic term for these changes, is linked to several diseases and can result in a variety of symptoms in the heart, nerves, muscles, pancreas, kidneys, and liver. It is conceivable that long-term difficulties in HIV-infected individuals would be caused by chronic infection, inflammation, and/or medications with negative effects on mitochondrial function. It has been reported that HAART-related side effects include a comprehensive number of clinical expressions of mitochondrial toxicity, which is a key concern for the selection and long-term adherence to a particular medication[140].

For instance, patients with HIV infection and receiving antiretroviral therapy have shown increased production of tumour necrosis factor (TNF), which has a direct impact on mitochondrial function. Nucleosides reverse transcriptase inhibitors have proven to be one of the major sources of HAART-induced mitochondrial toxicity which can be life-threatening and irreversible. The exact manner of development of these diseases remains known and differs in various NRTIs.[141]

NRTIs have the ability to inhibit DNA pol Y, which in turn leads to the inhibition of mitochondrial RNA expression which has been observed in several cells exposed to NRTIs.[142] This might happen due to mtRNA polymerase inhibition or a lack of cofactors required for mtDNA transcription[142]. Some NRTIs also effectively block a subset of unrelated mtDNA mitochondrial targets. As a result, AZT prevents the activity of the mitochondrial adenylate kinase and adenosine nucleotide translocator in isolated mitochondria. Additionally, AZT decreases OXPHOS while promoting oxidative stress (OS) and having a direct inhibitory effect on the electron transport chain[143,144]. Additionally, NRTIs result in a significant decline

in complex IV activity and a particular suppression of complex I[143,145.146]. Studies using AZT in vivo revealed OS in mtDNA, reduced expression of mitochondrial cytochrome b mRNA, and disturbed cardiac mitochondrial ultrastructure. It has also been proposed that NRTI-induced mitochondrial toxicity is caused by, mitochondrial reactive oxygen species (ROS) production but not all drugs in this category have the same level of toxic effect on the mitochondrial. This became a fact after several research was carried out in order to rank the level of toxicity of ART, depending on the cell line and the method used to measure mitochondrial dysfunction[147]. As a result, ddC is more toxic to other cell types like hepatocytes and peripheral blood mononuclear cells (PBMCs) while AZT and D4T are more hazardous to adipocytes. According to certain in vivo data, the ddC component of antiretrovirals (and likely the ddI component as well) rather than d4T or AZT mediates the loss of mtDNA in PBMCs in HIV-infected people.[147]

In addition to NRTIs, other anti-HIV medications like PIs and NNRTIs also appear to interfere with mitochondria[148]. Notably, neither PIs nor NNRTIs inhibits Pol-y nor do they lower the amount of mtDNA.

Studies conducted in vitro showed that nevirapine- or indinavir-treated mouse adipocytes had higher levels of mtDNA (NVP)[149,150]. Recent data reveals that these medication classes interact with mitochondrial targets that are important for controlling bioenergetics and apoptosis. Although the therapeutic impact of this interaction is unknown, it might be significant given the disorders that long-term HAART patients have. But most research indicates impacts specific to drugs rather than classes[151].

The rapid aging process of the population under ART and the occurrence of aging-related diseases are led to more investigation regarding mitochondrial DNA mutations and which induce mitochondrial dysfunctions.[152] These adolescents who have been under ART for 2-

10years, some from birth might have mitochondrial alterations that might lead to more chronic diseases and mitochondrial DNA-dependent alterations.

A study also supported the notion that HIV infection has immediate effects on mitochondria and that these changes are accompanied by immune cell activation and inflammation. According to research using a huMITOchip microarray, mitochondrial genes were negatively regulated in PBMCs and adipose tissue from HIV-infected patients who had not received treatment as compared to HIV-seronegative[153]. In comparison to HIV-seronegative controls and long-term non-progressors, a study showed elevated plasma mtDNA in acute HIV seroconverters and ART-naive individuals. Additionally, a study found a positive connection between plasma HIV RNA and plasma mtDNA[154]. Furthermore, it is important to note that both increases and decreases in mtDNA have been recorded in pathogenic conditions. Because there is no established method used to determine what amounts of mtDNA are defective, results from diverse HIV-infected patients were inconsistent[155-159].

In conclusion, the contribution of ART and HIV to mitochondrial dysfunction are still unclear, but, the underlying mechanisms remain partially understood. Genetics of the patient, the existence of mitochondrial diseases, and/or pathological conditions with impaired mitochondrial function are all significant factors that must not be overlooked as they can all amplify the negative effects associated with mitochondria[160].

There are numerous ways to determine how well mitochondria operate, including tests of the OXPHOS genes and enzymes, genes involved in the Krebs cycle, lipogenesis, measuring mitochondrial DNA damage by PCR, and so on. Thus, the outcomes could differ depending on the measurement employed[161].

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Chapter Three

Methodology

3.1 Research Design

This is a pilot comparative study, comparing values from HIV-positive patients who are on ART treatment to HIV-negative patients, using established quantitative polymerase chain reaction (qPCR) protocols to determine the mitochondrial DNA (mtDNA) copy numbers and damage. A convenient sampling method will be used because it is a pilot study being carried out to probe this knowledge gap among Adolescents living with HIV in Nigeria in preparation for an extensive study with references from the results gotten from this study.

3.2 Area of Study

The research population consisted of 30 adolescents living with HIV who are under combination antiretroviral therapy between the ages of 9years to 20years and 30 non-infected adolescents. These participants were recruited from the Nigerian Institute of Medical Research HIV clinic and the University College Hospital Ibadan in Ibadan North Local Government area longitude 7.3569^0N and latitude 3.8743^0E and longitude 6.5144, latitude 3.37103 respectively. Samples were analysed at the molecular laboratory in the department of the Center for Human Virology and Genomics in NIMR, Yaba-Lagos.

3.3 Study Population

The target population size for this study was 30 adolescents living with HIV who are under combination antiretroviral therapy from the ages of 9 years to 20years and 30 non-infected adolescents from the ages of 9years to 20years who came to the hospital for malaria treatment, genotype, and phenotype test. Patients were issued informed consent forms and participation was voluntary. These participants were selected in Lagos and Oyo state respectively.

3.4 Sample and Sampling Technique

A convenient sampling method was used, as this a pilot study carried out to probe the knowledge gap among Adolescent living with HIV in Nigeria, in preparation for an extensive study with references from the results gotten from this study. The sample size for the full study was calculated using the prevalence of Lipoatrophy in Southwest Nigeria, although:

$$N = Z_{\alpha}^2 Pq/d^2$$

Where: N = sample size

Zα = Z statistic for a 95% confidence level = 1.96

P = Prevalence of HIV patients with Lipoatrophy in Lagos = 0.26 (20).

q = Complementary probability = 1 - P = 1 - 0.26 = 0.74

d = Sample error or precision (5%) = 0.05

$$N = \frac{1.96^2 \times 0.26 \times 0.74}{0.05^2} = 296$$

3.5 Ethical Consideration and Consent Documentation

An application for ethical approval was made to the institutional review board (IRB) at the Nigerian Institute of Medical Research (NIMR) where analysis was carried out. After we received ethical approval, we proceeded to issue participant data forms and informed consent to individuals who volunteered to be part of this study. All information collected was treated with strict confidentiality. The medical history of the participants used for this study was got from the health centres and examined to ensure that they meet the inclusion and exclusion criteria.

3.6 Inclusion Criteria

Adolescents regardless of gender between the ages of 9 years and 20 years were recruited for this study. 30 positive patients under antiretroviral therapy and 30 who came in for regular medical check-ups or diagnoses. Only participants who gave consent and agreed for samples to be collected after being informed (written or verbal) will participate in this study.

3.7 Exclusion Criteria

The subjects below 9 years and above 20 years, those who did not pass the selection criteria in the study area, and those who did not give their consent were not recruited for the study. Subjects with sickle cells and down syndrome were excluded from this study.

3.8 Materials

Blood samples, Needles and syringes, cotton wool, tourniquets, disposable Pasteur, pipettes, calibrated micropipettes, normal saline, EDTA sample bottle, HIV test kits, disposable gloves, exercise books, biros, absorbent tissue paper, Disposable micropipettes range, Eppendorf tubes, microcentrifuge tube, Qiagen DNA extraction kits, vortex-genie pulse machine, Eppendorf centrifuge machine, Quant studio™ Real-time polymerase Chain Reaction thermal cycler (PCR), PCR disposable plates, foil seal, Qubit fluorometer machine, Qubit buffer, wash buffer, ethanol, spin column, collection tube, Eppendorf incubator, Cyfox Live Gel electrophoresis machine, agarose gel, TAE buffer, primers, probes, TaqMan master mix

3.9 Sample Collection Method and Storage

In conducting this study, 2mL of blood samples were collected from HIV negative and positive intravenously from individuals who volunteered and signed the informed consent forms. A total of sixty (60) samples for both negative and positive individuals were collected and stored in 5mL EDTA bottles at -80^{o}c while awaiting extraction.

3.10 Methods of Laboratory Analysis of Samples

3.10.1 DNA Extraction using Qiagen Kit from Blood

DNA extraction using QIAamp DNA kits (Qiagen), is intended for molecular biology applications, for isolation of genomic, mitochondrial bacteria, parasite, or viral DNA. It provides a silica-membrane-based nucleic acid purification from tissues, swabs, CSF, blood, body fluids, or washed cells from urine. In addition, it can be used for genomic and mitochondrial DNA can be purified from small amounts of fresh or frozen blood, tissue, and dried blood spots. With the aid of the manufacturer's guidelines, we were able to extract about 95% purified DNA with the following steps;

- 20µL of Qiagen protease (proteinase K) was pipetted into the bottom of a 1.5µL micro-centrifuge tube and a 20µL sample was added to the microcentrifuge tube.

  (NOTE: for samples with a volume less than 200µL, we added the appropriate volume of PBS (phosphate buffer saline) to balance the solution.
- We added 200µL buffer AL to the sample and mixed it by pulse vortex.
- It was incubated at 56◦c for 10 minutes
- The 1.5mL micro-centrifuge tube was centrifuged to remove droplets from the inside of the lid.
- 200µL of ethanol (absolute) was added to the sample and mixed again by pulse vortexing for 15 seconds.
- After mixing, the 1.5mL micro-centrifuge tube was briefly centrifuged to remove droplets from the lid.
- The mixture was taken and added into a spin column without wetting the rim and centrifuged at 8000rpm for 1 minute.
- The spin column was placed in a new collection tube, and 500µL Awl was added without wetting the rim.

- It was centrifuged at 8000rpm for 1 minute. The spin column was placed in a new collection tube, and 500uL of AW2 was added and centrifuged at 14,000rpm for 3mins.
- The spin column was placed in a new collection tube and spun at 14,000 rpm for 1min (spin drying).
- The spin column was placed in a clean 1.5mL micro-centrifuge tube, and 200mL Buffer AE was added and incubated at room temperature (15◦c -25◦c) for 1 minute then centrifuged at 8000rpm for 1 a minute, the result was stored at -20°c.

The process of quantification of the extracted DNA was carried out to quantify the quality of the DNA extracted.

3.10.2 DNA concentration via Qubit Fluorometer

The Qubit fluorometer was used for the quantification of the extracted DNA, this machine uses a fluorescence dye to detect the concentration of nucleic acids, the fluorescent does bind specifically to the analyte of interest thereby providing more accurate results. 1µL of DNA sample was added to 199µL of Qubit buffer and placed in the Qubit fluorometer machine which picks the fluorescence dye attached to the DNA and converts it to DNA concentration. Concentration for each sample was recorded. This was done through the process below.

3.10.3 Quantification of the extracted DNA

Following DNA extraction from whole blood, quantification is done using the Invitrogen Qubit-4 fluorometer® as follows:

- Qubit™ assay tube was set up for the required number of samples.
- The lid of one 0.5 mL Qubit™ assay tube was labeled for each specimen.
- 199 µL of the Qubit working solution was added to each tube.

- 1 µL of the extracted DNA was added to its corresponding labelled Qubit™ assay tube and vortex for 2-3 seconds. The final volume in each tube was 200 µL.
- The tubes containing the mix were incubated in the dark for 2 minutes at room temperature
- Samples were read in the Qubit fluorometer and recorded.

After the concentration of DNA samples was confirmed, we went further to carry out gel electrophoresis on the samples. Gel electrophoresis was done to check the integrity of DNA samples.

3.11 Gel Electrophoresis

Gel electrophoresis is a laboratory procedure, used to separate mixtures of DNA or RNA, or proteins according to their sizes. This process was carried out via the following process.

- Agarose gel (1.0%) was used to confirm the PCR amplification results.
- To prepare 1% (w/v) agarose solution, 1.0 g agarose was added to 100 mL 1X TBE to a glass flask.
- The mixture was microwaved carefully until boiling to form an agarose gel solution.
- The flask was swirled using padded gloved hands to prevent injury. This was repeated 3 times to ensure the gel had completely dissolved into the solution (No crystals were left in the gel solution by observing the gel solution against the light).
- The dissolved agarose solution was left to cool to 50 - 60°C at room temperature.

- 10 µL of GelRed (10,000X stock) was added to the 100-mL agarose solution for a 1X solution and mixed thoroughly.
- GelRed was used instead of the highly toxic Ethidium Bromide (EtBr) solution for staining DNA in agarose gel.
- The gel tray was set up and agarose gel was into the gel caster.
- Bubbles or debris were removed, and agarose was allowed to solidify at room temperature (approximately 30 minutes).

3.11.1 Sample Preparation for Agarose Gel Analysis

- 2 µL of 5-6X DNA loading buffer was added onto a parafilm.
- 5 µL of the sample was added to each tube or buffer spot on parafilm mixed with loading dye.
- A ready-to-use DNA mass ladder was used and only 7 µL of it was used.

3.11.2 Running the Gel Electrophoresis

- Once agarose had solidified, the comb was gently removed, and the prepared gel was lifted to an apparatus filled with 1X TBE buffer.
- 7 µL of each sample-loading buffer mix was added to wells (With Molecular Marker in the first well and the pipette tip was changed with each sample to prevent cross-contamination).
- The gel was run at 100V for 30-45 minutes to migrate gel loading dye front 4-5 cm.
- The loaded material was checked if it ran toward the positive (red) end of the gel box sufficiently to visualize the bands and identify specific weights.
- The gel was visualized on a bioimaging system and photographed.

- The test sample was considered void when:

    a. The major product is not the appropriate size.
    b. The major product is the correct size 1.1 kb, but too much smearing exists.
    c. There is no PCR product visible.

After DNA sample integrity has been checked, the proportion of deleted mtDNA was determined using TaqMan real-time PCR assay. The reactions were performed in twenty microliters volume, five microliters of DNA sample were amplified separately with ND1 and ND4, and in a set of wells DNA samples of five microliters were amplified with the B2M primers ad probes combinations. Probes were designed with an MGB moiety. The sequences for both primers and probes are mentioned below. To minimize pipetting error, pipette tips were flushed out 20 times. Reactions were done in replicates of three on a single real-time plate and total reactions were carried out on two plates resulting in six replicates per sample.

3.12 Primer Design

Primers used were made to specifically target ND1 and ND4 genes in the mitochondrial genome and B2M in the nuclear genome as it is to serve as the standard for comparison to calculate the level of deletion of the mitochondrial genome.

Table 3.1: Primer and probes. Source[3]

Assay	Mitochondrial DNA deletion assay.
Forward primer(s)	MT- ND1 5'-CCCTAAAACCCGCCACATCT-3'
Reverse primer(s)	MT- ND1 5'- ACCTTAGCTCTCACCATCGCTC-3'

Probe(s)	MT- ND1 probe VIC 5'-CCATCACCCTCTACATCACCGCCC-3'-MGB
Assay conditions	2 min at 50C, 10min at 95C then 40cycles of 15s at 95C and 1min at 60C.[1]
Comments	Reactions in 20ml volumes. Probes were designed with a non-fluorescent quencher and MGB moiety. PCR primer conc -300nM and probe -100nM[1] Multiplex MT ND1/MT ND4 TaqMan real-time PCR assay.
Forward primer(s)	MT- ND4 5'- CCATTCTCCTCCTATCCCTCAAC-3'
Reverse primer(s)	MT- ND4 5'- AAATATAGTTTAACCAAAACATCAGATTGTG-3'
Probe(s)	MT- ND4 probe FAM 5'-CCGACATCATTACCGGGTTTTCCTCTTG-3'- MGB
Assay conditions	2 min at 50C, 10min at 95C then 40cycles of 15s at 95C and 1min at 60C.[2]
Comments	Reactions in 20ml volumes. Probes were designed with a non-fluorescent quencher and MGB moiety. PCR primer conc -300nM and probe -100nM[2] Mitochondrial DNA copy number singleplex nuclear DNA
Forward primer(s)	B2M 5'- CCAGCAGAGAATGGAAAGTCAA- 3'
Reverse primer(s)	B2M 5'- TCTCTCTCCATTCTTCAGTAAGTCTAACT -3'
Probe(s)	B2M 6FAM -5'- ATGTGTCTGGGTTTCATCCATCCGACA -3' MGB
Assay conditions	2 min at 50C, 10min at 95C then 40cycles of 15s at 95C, and 1min at 60C.

Comments	Final B2M PCR primer conc -300nM and probe -100nM. Each 20ml B2M reaction was supplemented with 3nM MgCl2.[3]

3.12.1 Master Mix Preparation

The master mix used for this analysis was prepared using the following measurements.

Qiagen and Invitrogen Superfi Platinum

[Each primer= 300nM and Each probe= 100nM]

Measurements used for ND1 and ND4 multiplex reaction.

Table 3.2: Master mix measurements. **Source**[2,3]

Component	**1X(µl)**	**9X**	**50X**
Water	3.00	27.00	150.00
MMx	12.50	112.50	625.00
1-ND1- forward (10µM)	0.75	6.80	37.50
1-ND1 reverse (10µM)	0.75	6.80	37.50
1-ND1 probe (10µM)	0.25	2.30	12.50
2-ND4- Forward (10µM)	0.75	6.80	37.50
2-ND4 Reverse(10µM)	0.75	6.80	37.50
2-ND4 Probes (10µM)	0.25	2.3	12.50
ROX	0.25	2.3	12.50
50mM MgSO4 (put 1.5mM)	0.75	6.8	37.50

Vol MMx per well	20.00
DNA (Total 50 ng;10ng/µl)	5.00

Qiagen and Invitrogen Superfi Platinum

[Each primer= 300nM and Each probe= 100nM]

Table3.3: Representation of the measurements used for B2M singleplex reaction. **Source**[2,3]

Component	**1X(µL)**	**9X**	**50X**
Water	4.75	42.8	237.5
Mmx	12.50	112.50	625.00
1-B2M-forward (10µm)	0.75	6.8	37.50
1-B2M-Reverse (10µm)	0.75	6.8	37.50
1-B2M-Probes (10µm)	0.25	2.3	12.50
Rox	0.25	2.3	12.50
50Mm mgso4 (put 1.5mm)	0.75	6.8	37.5
Vol mmx per well	20.00		
DNA (Total 50ng;10ng/µl)	5.00		

3.13 Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a technique used in the molecular laboratory to amplify a particular segment of the DNA and generate billions of copies of the DNA sequence. The basic principle of a PCR machine is a chain reaction as one DNA molecule is used to create two copies and then four copies then eight copies and so forth. This process is carried out in a mixture containing DNA template (extracted DNA), Taq polymerase, primers, probes (florescent dye), and master mix, which are placed under various temperature conditions from 0°C and 100°C. We targeted two points on the mitochondrial DNA (ND1 and ND4) and on the nuclear DNA (B2M) and the process was repeated for about 35 cycles. The real-time PCR machine used allowed programming of the temperatures and the number of cycles required. The procedure was carried out through these processes.

3.13.1 Procedure

a. **Denaturation**: DNA template was heated to a temperature of about 90°C which allowed the weak hydrogen bond between nucleotides to break, causing DNA strands to separate, creating two new single strands.
b. **Annealing**: Primers annealed to their complementary newly formed single DNA strands as temperature was reduced to about 50°C.
c. **Extension:** Temperature was increased to about 72°C and enzymes were activated and allowed to act and complementary DNA strands were created at the end of the annealed primers.

After 30 cycles new copies of DNA strands would have been made. Using real-time PCR, analysis of the products can be carried out as it uses a florescent dye that reacts with the amplified DNA which can also be measured by the machine.

3.14 Statistical Analysis

Data collected was entered in Microsoft Excel sheet and statistical analysis was done using statistical package for social sciences (SPSS) software. Descriptive analysis was used to present prevalence and frequencies of outcomes. The mean values were calculated and Chi-square was used to determine the association between two categorical variables. P values ≤ 0.05 at 95% confidence interval was considered significant.

Endnotes

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[4.] Qubit™ 3.0 Fluorometer from ThermoScientific : Get Quote, RFQ, Price or Buy, news-medical.net. Available Online: https://www.news-medical.net/Qubit-3-Fluorometer-from-Thermo-Scientific. 2022

[5.] Pin von Threedotts design auf Ritssie | Medizinisches design, Design wettbewerb, Industriedesign (pinterest.com), Available online: https://www.pinterest.com/pin/305752262173305375/. 2020

Chapter Four

Results and Discussion of Findings

4.1 Gender of Participants

The total number of adolescents that participated in this study was 60. Thirty (30) adolescents who were HIV positive and 30 who were negative were recruited. The age range of adolescents who were HIV positive was from 9-22 years. However, there was no statistical difference between the mean age of participants that were HIV as compared to those who were HIV negative as P> 0.05. Table 4.1 describes the gender of the participants stratified between adolescents who are HIV positive and those who are negative

Table 4.1: HIV-Status Gender Per Sample Table. Source: Author's Field work, 2022

		Gender		Total
		F	M	
HIV- Status	Negative	15	15	30
	Positive	11	19	30
Total		26	34	60

p> 0.05 Hence no significant difference

4.2 Age Difference

Age was normally distributed among the participants of the study. The mean age difference between HIV positive and HIV negative cohorts did not reach a significant level, therefore there was no bias in the age distribution (P>0.05).

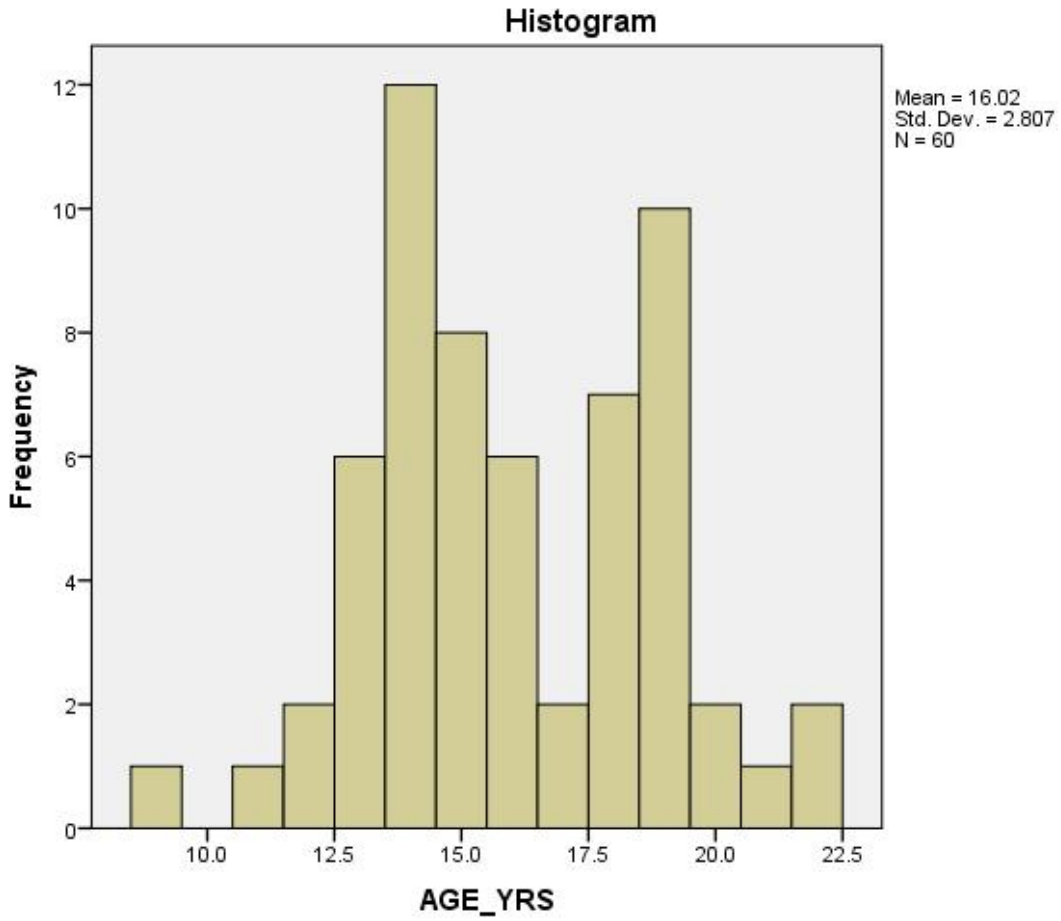

Figure 4.1: Age Distribution of Participants.

Source: Author's Field work,2022

Table 4.2: Mean Age of Participants Stratified According to HIV Status

	HIV- Status	N	Mean	Std. Deviation
Age-Years	Positive	30	15.87	3.235
	Negative	30	16.17	2.350

Source: Author's Field work,2022

4.3 Duration of Antiretroviral Therapy

Among adolescents who are HIV positive, under antiretroviral therapy, 53.33% have been exposed to ART for a period of 2-5years while 20% have been exposed to ART for more than 10 years. See (Figure 4.2).

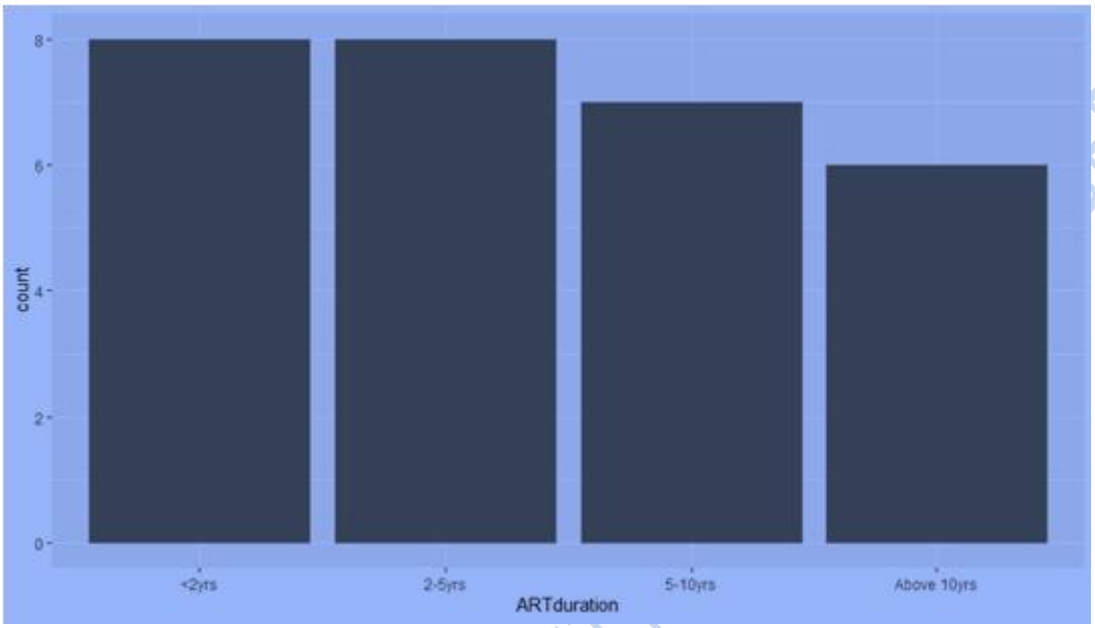

Figure 4.2**:** ART duration in years of participants

Source: Author's Field work,2022

4.4 Types of Antiretroviral Therapy

Participants who are HIV positive and under antiretroviral therapy were under different regimes ranging from TLD combination (Tenofovir/ lamivudine/ dolutegravir) at 61.67% to ATV/r/ABC/3TC combination (Atazanavir/ ritonavir/ abacavir/ lamivudine) at 16.67% while a lower number of participants were under DTG/ABC/3TC combination (Dolutegravir /abacavir/ lamivudine), LPV/r/AZT/3TC combination (Lopinavir/ ritonavir/ Azidothymidine / lamivudine), AZT/3TC/EFV combination (Azidothymidine/ lamivudine/ efavirenz).

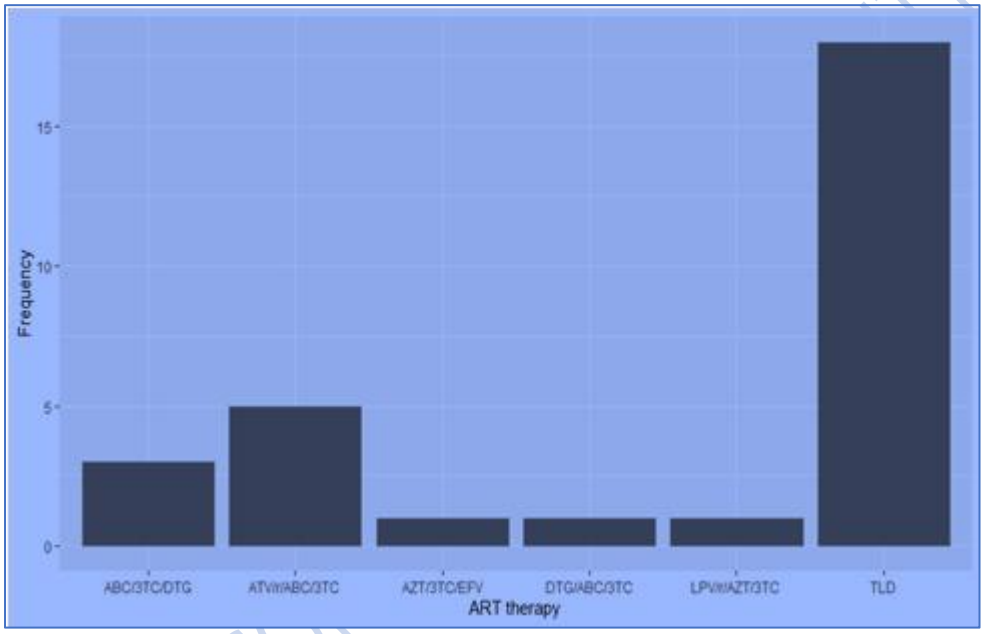

Figure 4.3: Distribution of Antiretroviral Therapy

Source: Author's Field work,2022

4.5 DNA Concentration using Qubit Fluorometer

The highest DNA concentration among the HIV-positive samples was 44.6 ng/µL and the lowest DNA concentration was 8.32 ng/µL. The mean value was 18.19±8.92ng/ µL. The highest DNA concentration from HIV-negative samples was over 600 ng/ µL and the lowest DNA concentration was 0.62 ng/ µL. The mean value was 27.31±144.59ng/ µL

Table 4.3: Positive Samples DNA Concentration

Positive samples	Average DNA concentration
C001	10.4
C002	10.5
C003	21.6
C004	11
C005	12.3
C006	18.7
C007	10.9
C008	17.8
C009	44.6
C010	8.32
C011	29
C012	9.78
C013	8.74
C014	18
C015	12.6
C016	9.98
C017	26
C018	14.6
C019	17.5
C020	15.9
C021	11.4
C022	17.8
C023	27.4
C024	26.6
C025	21.2
C026	12.2
C027	18.5
C028	42.2
C029	24.4
C030	15.9

Source: Author's Field work, 2022

Table 4.4: Negative Samples DNA Concentration

Negative Samples	DNA Concentration
S001	11.9
S002	5.52
S003	18.9
S004	22.8
S005	0.62
S006	56.8
S007	35.2
S008	Above 600
S009	11.5
S010	34.4
S011	13.7
S012	18.6
S013	18.5
S014	13.2
S015	18.1
S016	118
S017	39.6
S018	61
S019	23
S020	15.3
S021	12.9
S022	Above 600
S023	58.6
S024	25.6
S025	11.3
S026	39.8
S027	12.8
S028	23.4
S029	17.3
S030	26.4

Source: Author's Field work, 2022

4.6 Gel Electrophoresis

DNA integrity for samples collected from both groups was accessed using gel electrophoresis. The DNA integrity for all participants irrespective of the groups was confirmed and represented below in Figures 4.4 and figure 4.5

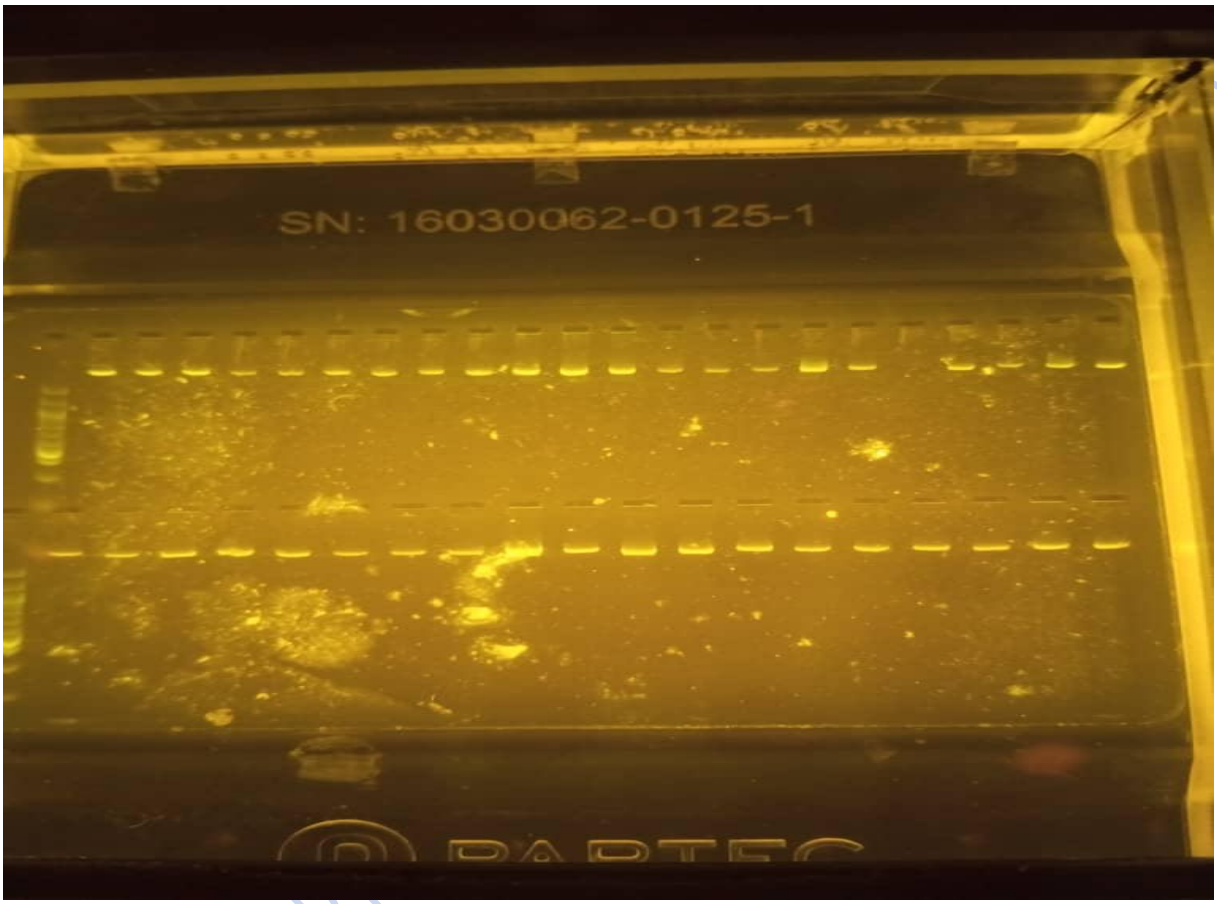

Figure 4.4: A Pictorial Representation of Positive Gel Electrophoresis

Source: Author's Field work,2022

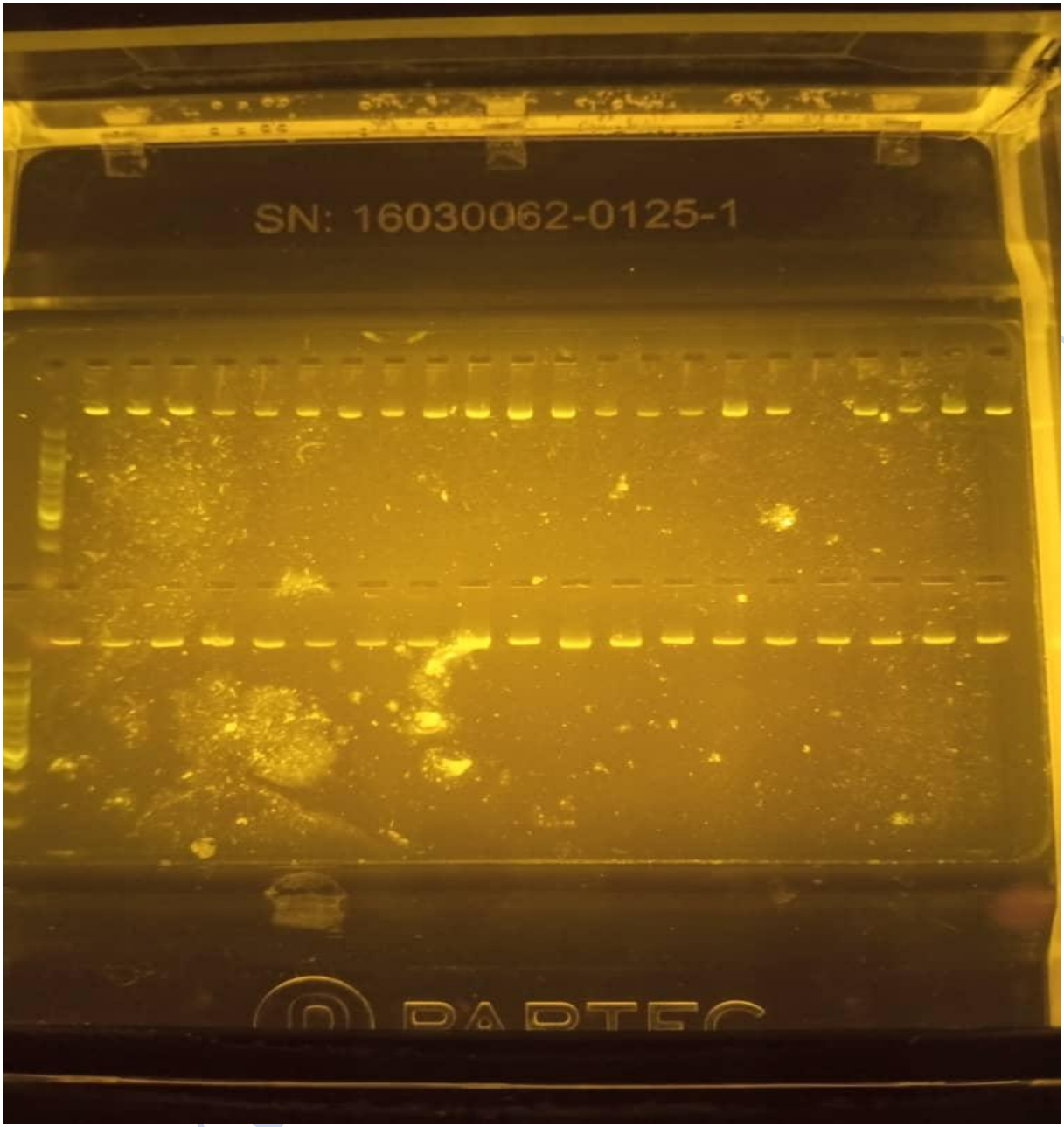

Figure 4.5: A Pictorial Representation of Negative Gel Electrophoresis.

Source: Author's Field work,2022

4.7 Amplification of ND1, ND4, and B2M Gene

Quantitative data were extracted from Quant studio™ using Microsoft excel and further analyzed using Statistical Package for the Social Sciences (SPSS). We assigned distinctive codes to each participant's record to protect their privacy. The real-time PCR assay was successful in all the runs and figures 4.6 and figure 4.7 are amplication charts for some of the runs. The total mean ct for the Nd1 gene among the HIV-positive cohort was 21.11 ±1.75, the total ct for the positive Nd4 gene was 18.84 ±0.05, and 23.76 ±0.7 for the B2M gene. For the HIV-negative cohorts, the total mean CT for Nd1gene was 19.88 ±1.46 and the total mean ct for the Nd4 gene was 18.34± 0.96 and the B2M gene total mean ct was 24.01 ±1.06.

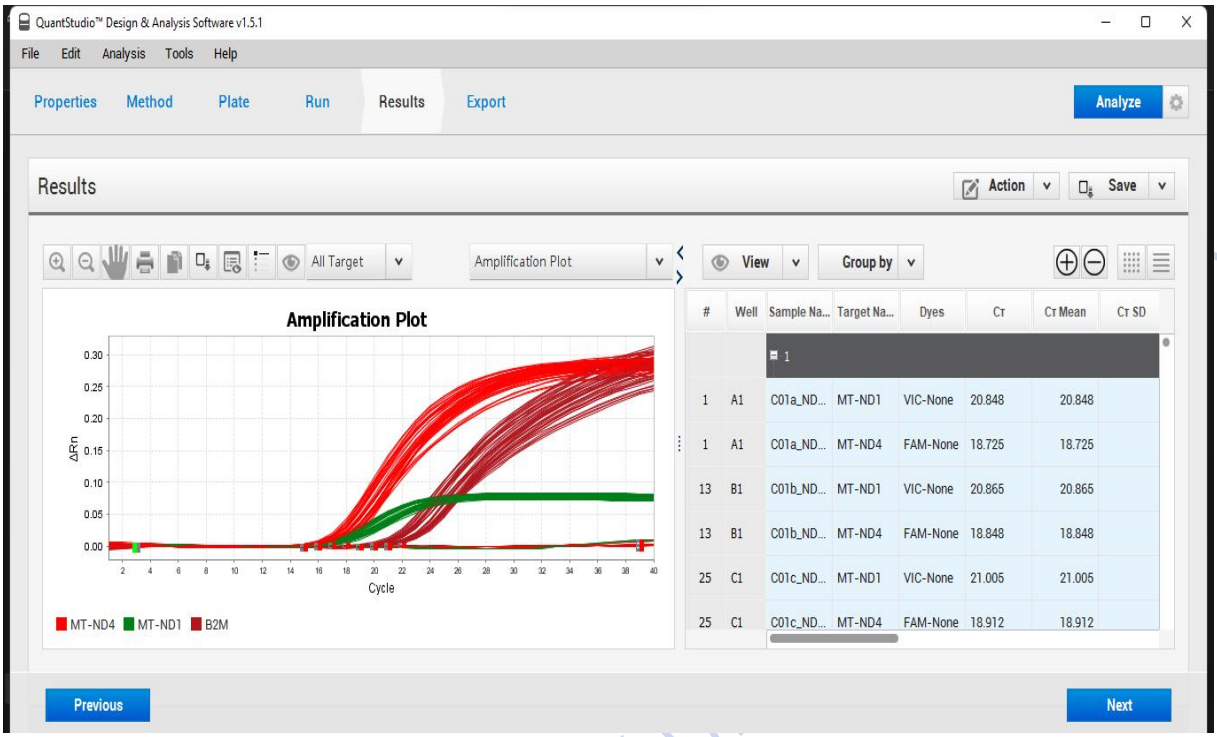

Figure 4.6: Pictorial representation of amplification plot of gene targets, showing dyes, and CT values in Quant studio 5 machine.

Source: Author's Field work, 2022

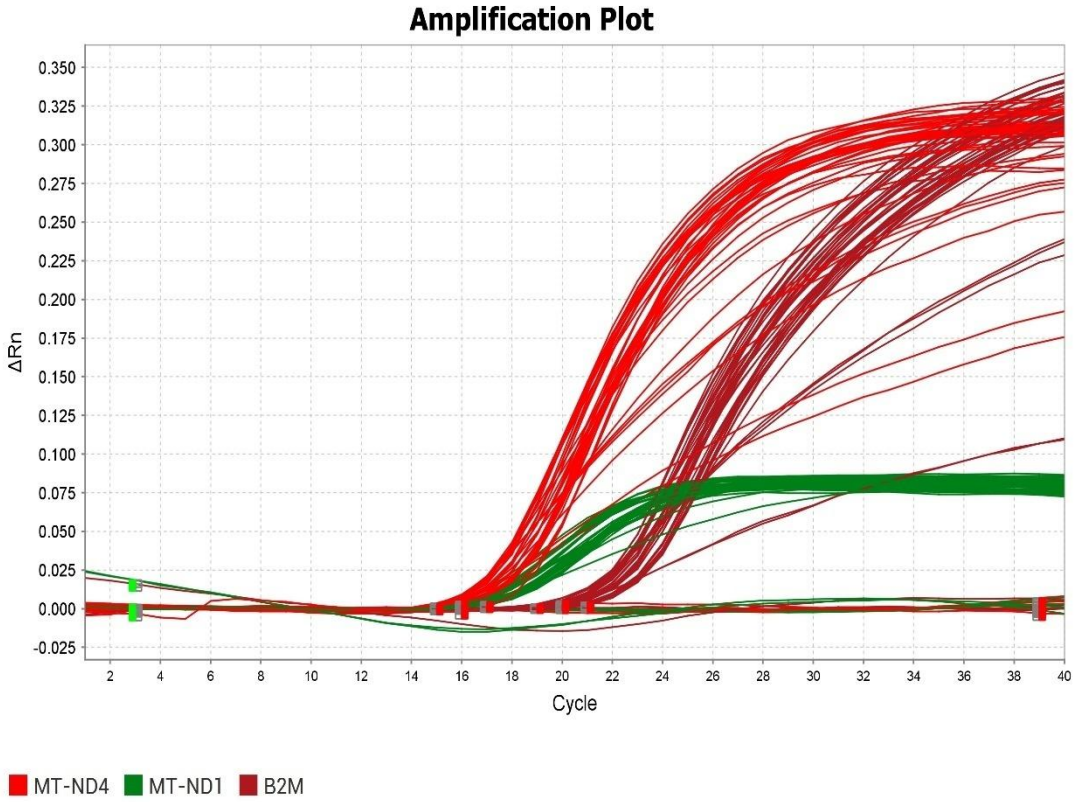

Figure 4.7: Pictorial representation of amplification plot of gene targets, and wells in Quant Studio 5 machine.

Source: Author's Field work, 2022

4.8.1 Assessment of Mitochondrial Deletion in HIV-Positive and HIV-Negative Cohorts

The mean deletion for both positive and negative cohorts was 25.84±3.96 and 35.26±9.55 respectively. However, there is a significant difference between the average mean deletions between HIV positive and HIV negative cohorts at $p<0.05$.

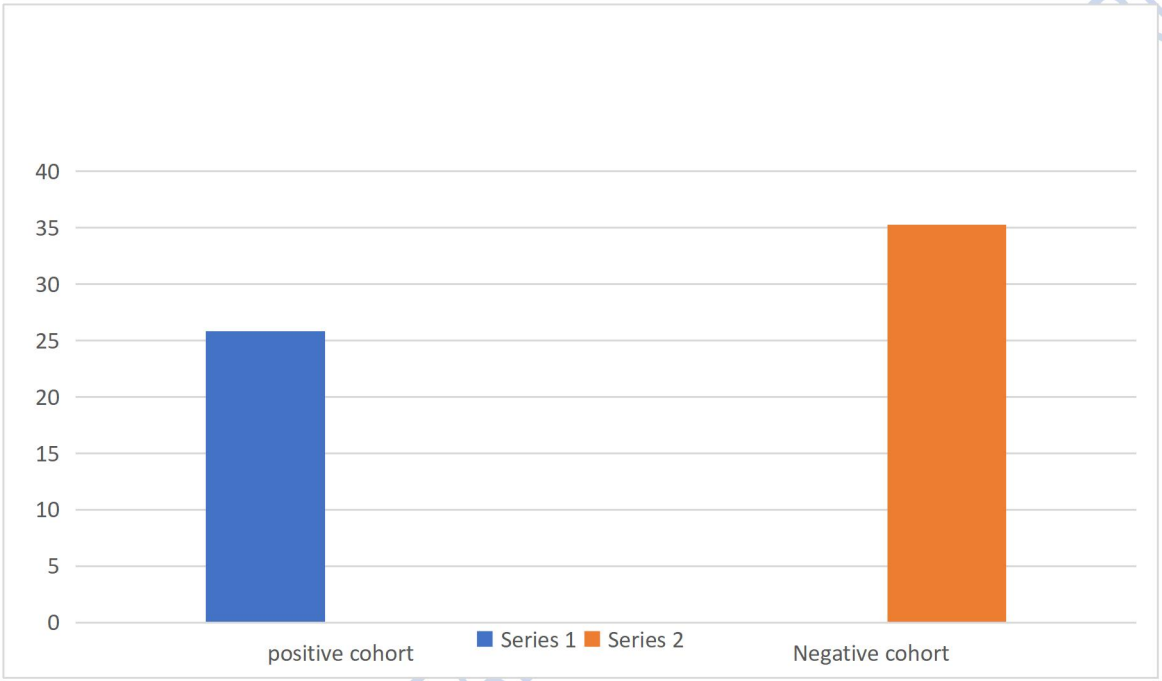

Figure 4.8: Deletions in both HIV-positive and negative cohorts

Source: Author's Field work, 2022

4.9 Assessment of Mitochondrial DNA Copy Number in HIV-Positive and HIV-Negative Cohorts

The mean mitochondrial copy number in both positive and negative cohorts were 87.87±1.62 and 53.18±30.52 respectively. There was a significant difference between the average mean mitochondrial DNA copy number between HIV positive and HIV negative cohorts at P<0.05.

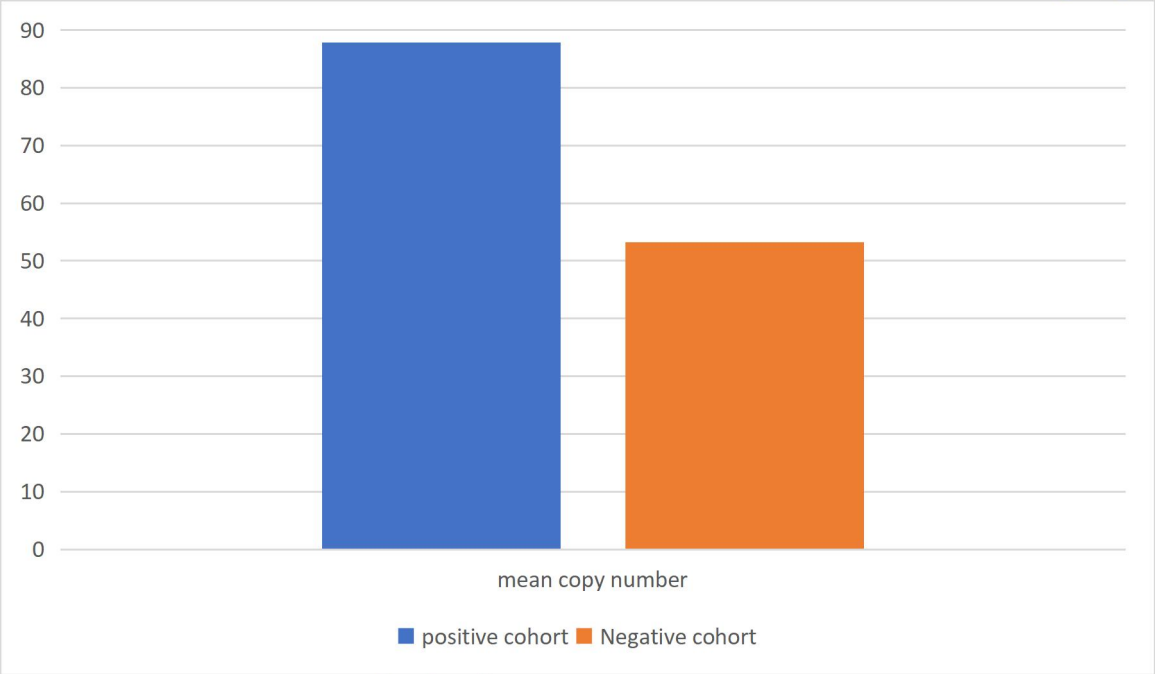

Figure 4.9: Mean Mitochondrial DNA copy numbers in both cohorts

Source: Author's Field work, 2022

Table 4.10: Positive Samples Multiplex and Singleplex Reaction Using PCR.

Source: Author's Field work,2022

Sample ID	ND1 (multiplex)	ND4 (multiplex)	B2M (singleplex)	Average Mean Δct	Average mean del	Average mean copy number
C001	21	18.88	23.93	2.12	22.29	87.74
C002	21.26	19.13	24.2	2.13	22.2	87.87
C003	21.01	18.89	23.11	2.12	22.38	90.91
C004	21.41	19.2	24.28	2.22	20.45	88.2
C005	21.58	19.51	24.7	2.07	23.43	87.4
C006	20.2	18.29	23.23	1.91	27.5	86.97
C007	21.2	19.17	24.33	2.03	24.31	87.16
C008	19.98	17.93	22.67	2.05	24.09	88.15
C009	20.75	18.76	23.49	2	25.3	88.36
C010	21.41	19.64	24.32	1.78	31.86	88.04
C011	19.98	18.09	22.88	1.89	28.03	87.32
C012	21.09	19.21	24.45	1.88	28.51	86.24
C013	20.18	18.35	24.29	1.83	29.95	83.07
C014	20.47	18.6	23.36	1.87	28.99	87.61
C015	21.2	19.98	23.91	1.82	30.69	88.66
C016	21.16	19.18	23.96	1.98	25.68	88.31
C017	19.95	17.99	22.85	1.96	26.22	87.3
C018	20.01	18.35	23.43	1.73	34.94	85.61
C019	30	18.68	23.3	2.32	19.31	90.15
C020	20.64	18.64	23.7	2.00	25.93	87.12
C021	21.01	18.97	24.06	2.04	24.17	87.31

C022	20.25	18.26	23.28	1.99	25.28	87.01
C023	21.32	19.31	23.54	2.01	24.99	90.57
C024	-	19.34	26.18			
C025	20.54	18.71	23.20	1.83	30.85	88.49
C026	21.06	19.11	23.92	1.95	26.38	88.02
C027	21.36	19.46	24.14	1.9	27.94	88.49
C028	20.34	18.41	23.69	1.92	27.2	85.82
C029	20.67	18.64	22.86	2.03	24.42	90.4
C030	21	18.54	23.41	2.52	16.02	89.97

Table 4.6: Negative Samples Singleplex and Multiplex Reaction Using PCR

Source: Author's Field work,2022

Sample ID	ND1 (multiplex)	ND4 (multiplex)	B2M (singleplex)	Average Mean ct	Average mean del	Average mean copy number
s001	21.45	19.66	25.06	1.78	31.76	85.55
s002	21.57	19.61	25.78	1.97	25.88	83.69
s003	19.66	17.8	23.58	1.86	29.42	83.39
s004	19.97	18.28	24.38	1.69	35.27	81.91
s005	23.7	21.94	26.96	1.76	32.31	87.92
s006	19.07	17.48	23.43	1.59	39.6	81.37
s007	20.69	18.91	24.49	1.78	33.54	84.51
s008	19.53	17.9	23.62	1.63	37.55	82.67
s009	19.9	18.11	23.81	1.79	31.33	83.59
s010	20.08	18.55	24.86	1.53	42.62	80.77
s011	18.83	17.2	24.9	1.64	37.34	75.64
s012	19.79	18.16	23.91	1.63	37.98	82.77
s013	19.23	17.77	23.53	1.46	47.17	81.74
s014	20.32	18.74	24.15	1.58	40.3	84.13
s015	20.69	18.53	24.86	2.16	26.1	83.23
s016	21.52	18.91	24.82	2.61	14.74	43.49
s017	14.55	18.93	24.37	-4.39	24.63	28.76
s018	18.35	16.9	21.01	1.44	48.43	19.57
s019	19.96	18.3	23.6	1.66	36.33	21.94
s020	18.54	16.97	22.87	1.57	40.58	21.3
s021	20.63	18.03	23.37	2.65	14.22	20.72
s022	19.11	17.63	22.85	1.43	48.82	21.42
s023	19.71	18.01	24.09	1.71	34.59	22.38
s024	20.44	18.85	24.24	1.58	40.71	22.65
s025	20.14	18.65	24.11	1.48	45.85	22.63
s026	20.15	18.67	24.25	1.49	45.21	22.77

s027	19.79	18	24.44	1.79	31.49	21.65
s028	19.18	17.72	22.96	1.47	46.5	21.49
s029	21.16	18.68	23.55	2.48	16.25	21.06
S030	18.76	17.21	22.34	1.56	41.31	20.78

Source: Author's Field work, 2022

4.10 Correlation Between Mitochondrial Copy Number and Mitochondrial Deletions in the Study

There is a significant negative correlation between mitochondrial copy number and mitochondrial deletions as **R**= -3.96 and P=0.002.

4.11 Correlation Between Mitochondrial Copy Number and Duration of Exposure To Antiretroviral Therapy

The correlation coefficient between mitochondrial copy number and duration of exposure to ART among adolescents living with HIV was 0.031. However, the correlation did not reach a statistically significant level.

Chapter Five

Conclusion

5.1 Discussion

Assessment of mitochondrial DNA deletions and copy numbers have been used as tools to assess the progression of diseases, metabolic breakdown, and the effect of exposure to infections and chemical agents such as drugs. This study, therefore, evaluates the level of mitochondrial toxicity among adolescents living with HIV and exposed to antiretroviral therapy.

In this study, we found a higher amount of mitochondrial copy number in adolescents who are HIV positive as compared to those who are HIV negative. However, Jing Sun et.al found mitochondria DNA copy numbers in people living with HIV below the age of 50 years to be similar to people living without HIV unlike Patients above 50 years living with HIV with a lower mitochondrial DNA copy number. We believe our findings support this conclusion as adolescents under ART are below 50 years. Also, Villiers et.al recorded patients living with HIV on ART who are strictly adhering to treatment have lower rates of viral suppression.

These findings of higher mitochondrial DNA copy number in positive HIV adolescents could be due to the early start-up of antiretroviral therapy and the body repair mechanisms working more to replace affected mtDNA

There is a low deletion level among HIV-positive adolescents as a result the body repair mechanisms working more to replace affected mtDNA this would help support metabolic activities thereby reducing oxidative stress.

However, Jing Sun, et.al[1] found out that mitochondrial deletions are not pronounced among people living with HIV until when they are above 50 years of age. Therefore, this could also be one of the reasons why there were no effective deletions in the mitochondrial DNA.

Furthermore, the new antiretroviral regimes have been reported to have a higher safety profile as compared to the older group of antiretroviral drugs. Therefore, most adolescents may be exposed to a safer combination of antiretroviral which is of good and safe metabolic activities.

5.2 Recommendations

1. It is therefore recommended that a similar study should be carried out on a larger population of adolescents infected with HIV and under antiretroviral therapy using molecular diagnostic methods searching for the genotoxic effect of prolonged usage of antiretroviral therapy.
2. Supplements should be added to the diet of patients infected with HIV as it boosts the immune system thereby improving the effect of antiretroviral therapy.

5.3 Contribution to Knowledge

The early diagnosis and treatment of HIV infected patients with new ART, helped in building the immune system of the patients and decreasing level of mitochondrial DNA deletions.

Mitochondrial DNA copy number was seen to be at a higher level as compared to what was initially proposed, due to the body repair mechanisms working more to replace affected mtDNA in HIV positive patients.

5.4 Suggested Area of Research

This study should be carried out on a larger cohort covering the exact cause of genomic damage between HIV patients under Antiretroviral therapy.

Furthermore, more studies should be carried out to establish an assertion on the source of mitochondrial copy number deletions in patients infected with HIV and under antiretroviral therapy.

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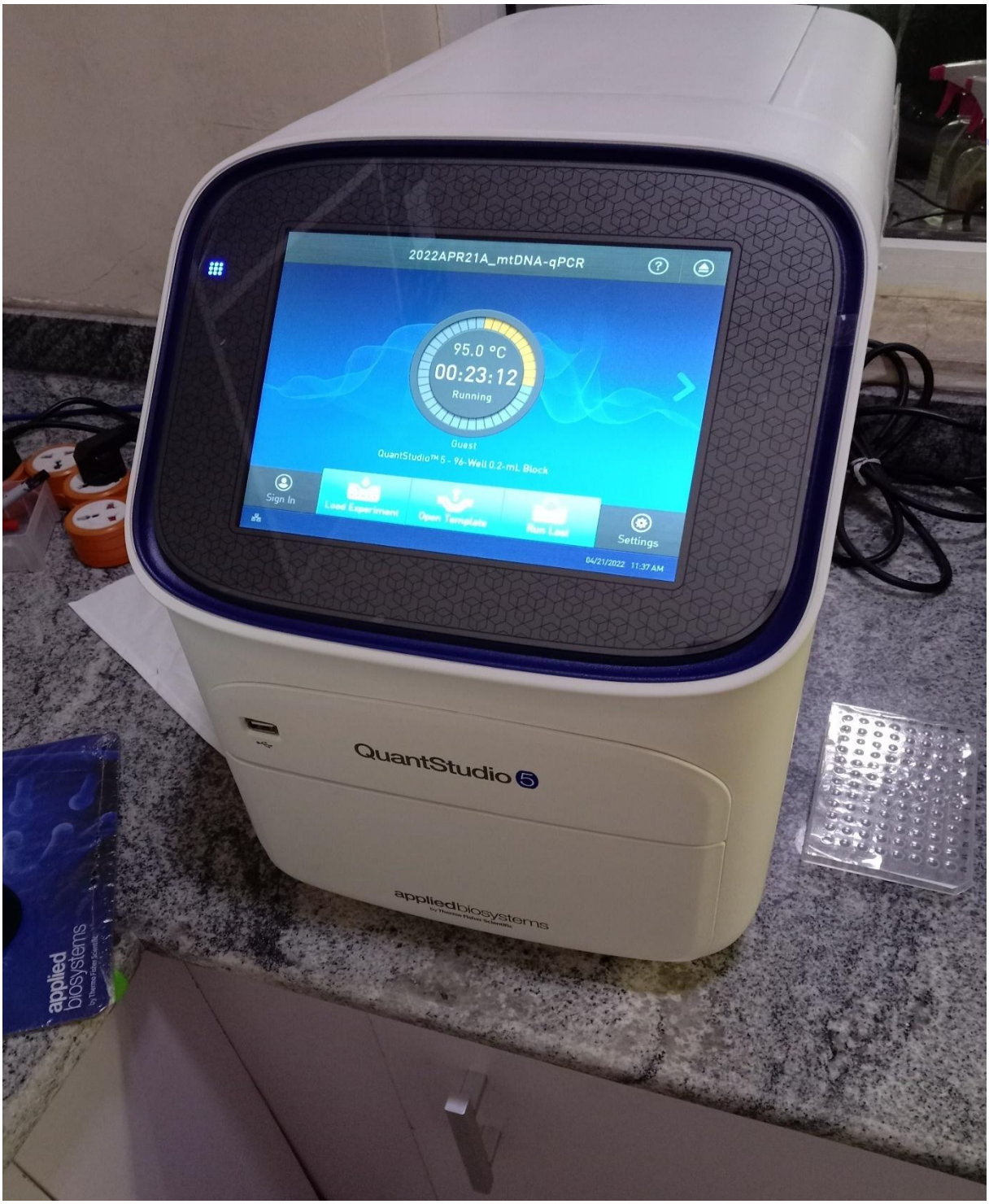
Appendix I

Master mix preparation.

Source:Author's Field work, 2022

Appendix II

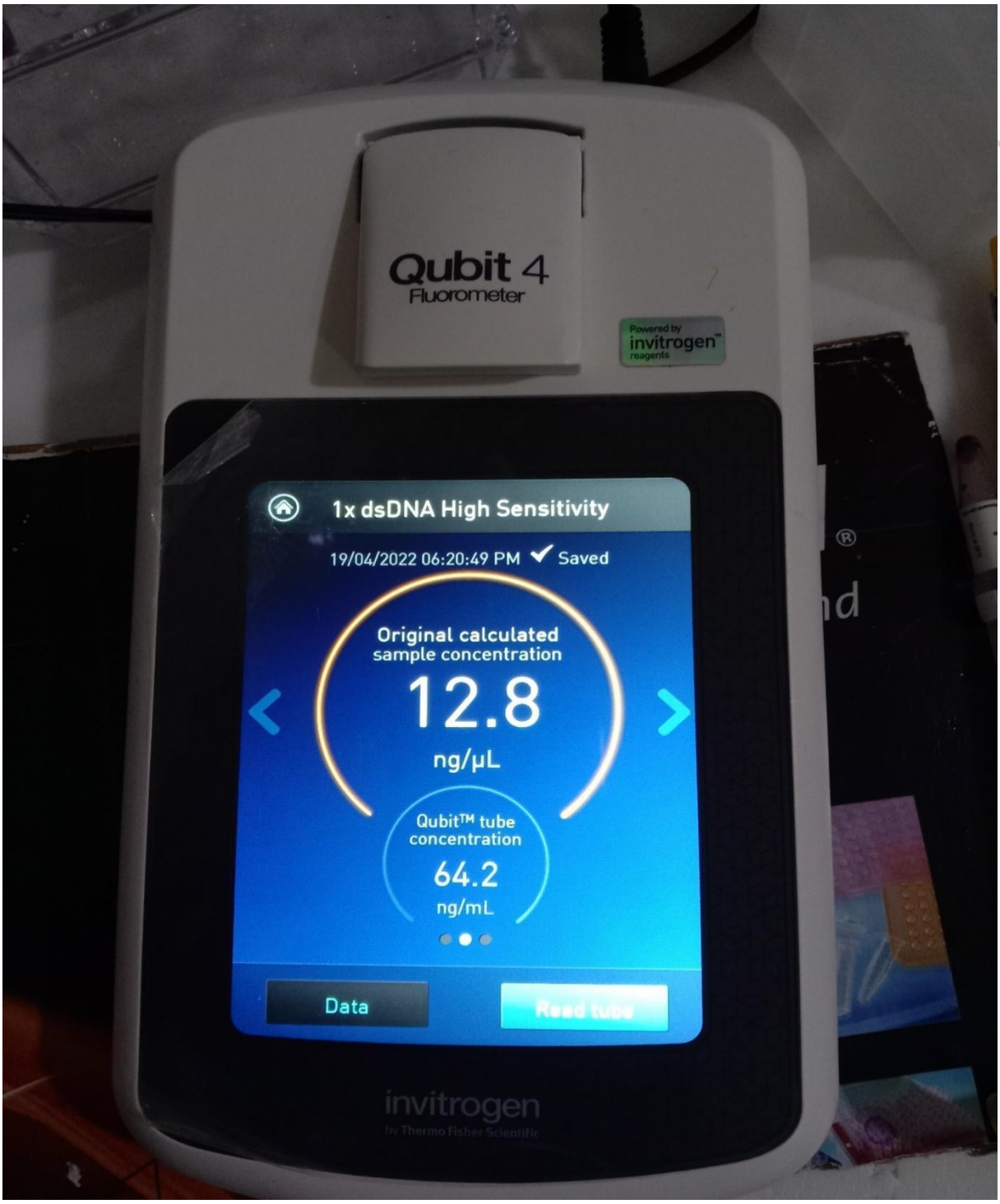
Quantstudio Realtime PCR in the process of amplification



Source:Author's Field work, 2022

Appendix III

Qubit 4 fluorometer in the process of DNA concentration quantification



Source:Author's Field work, 2022

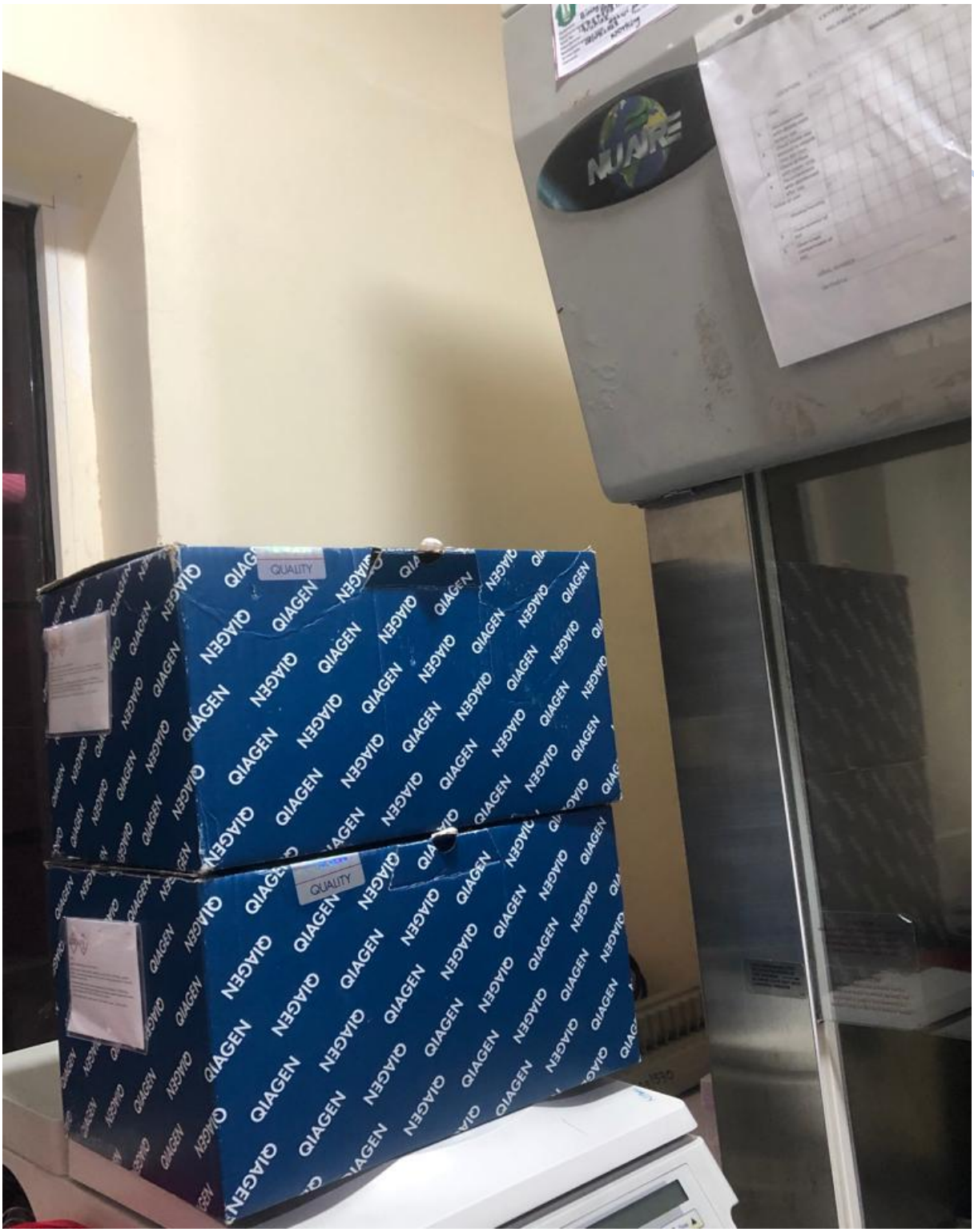
Appendix IV

Pulse voltex machine

Source:Author's Field work, 2022

Appendix V

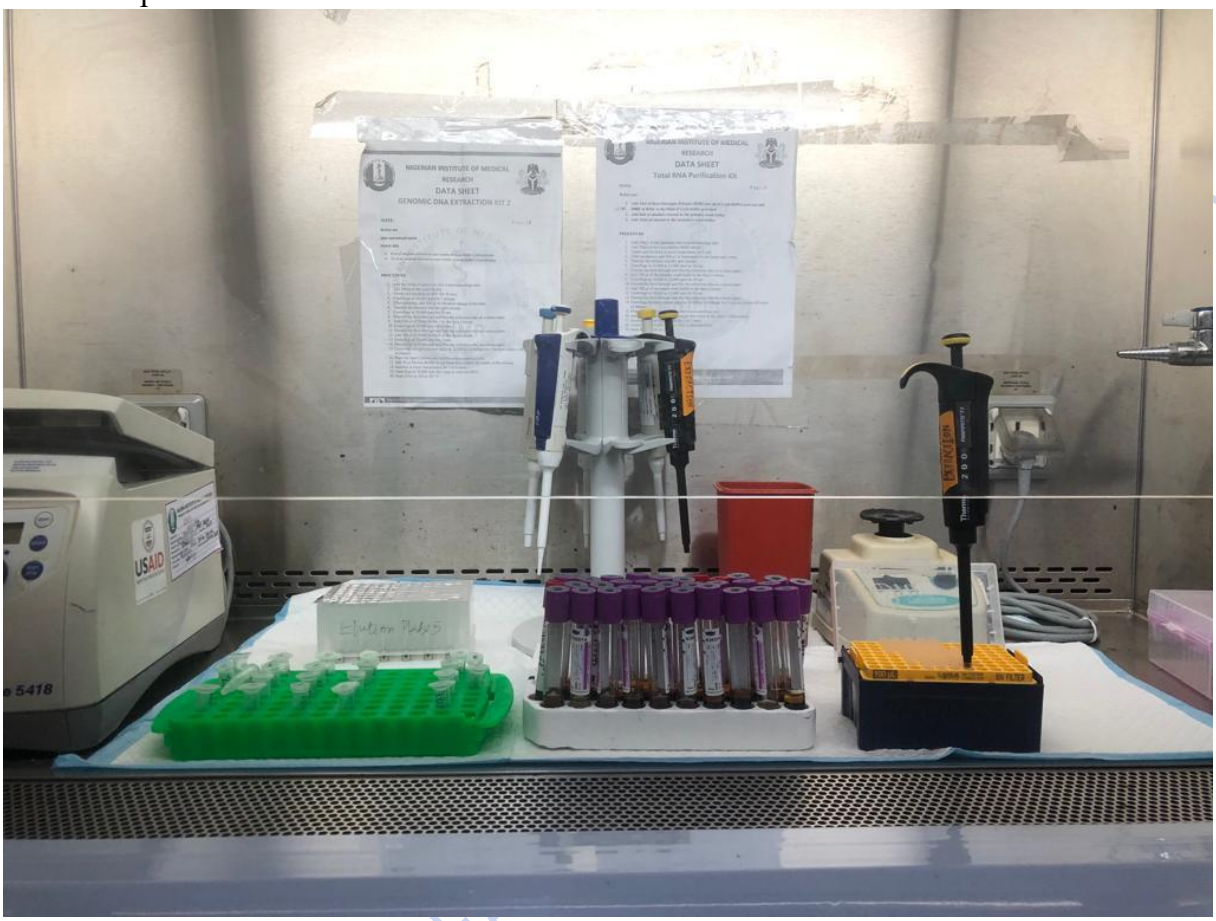
Qiagen DNA extraction
kits



Source: Author's field work,2022

Appendix VI

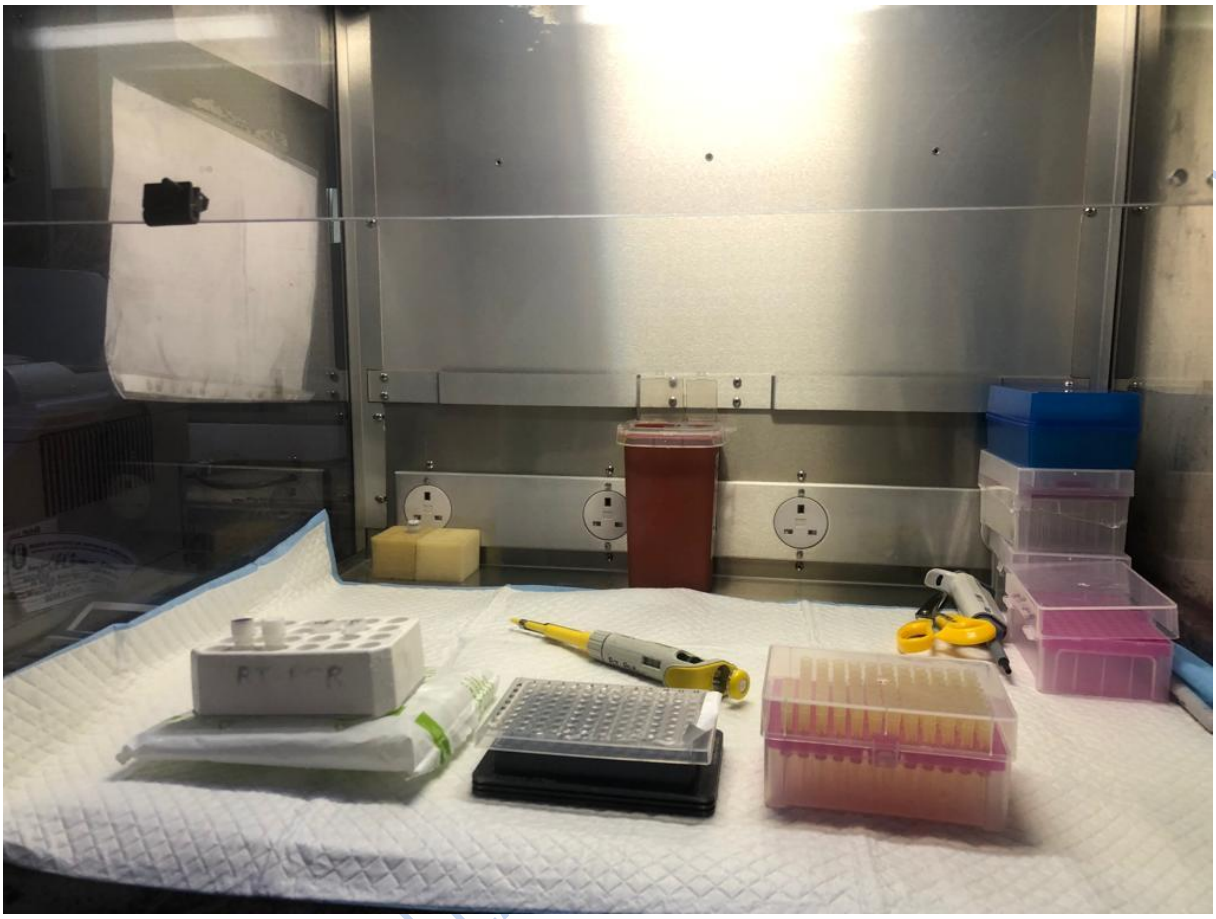
Blood samples before DNA extraction



Source: Author's field work,2022

Appendix VII

Polymerase chain reaction plates before analysis



Source: Author's field work,2022

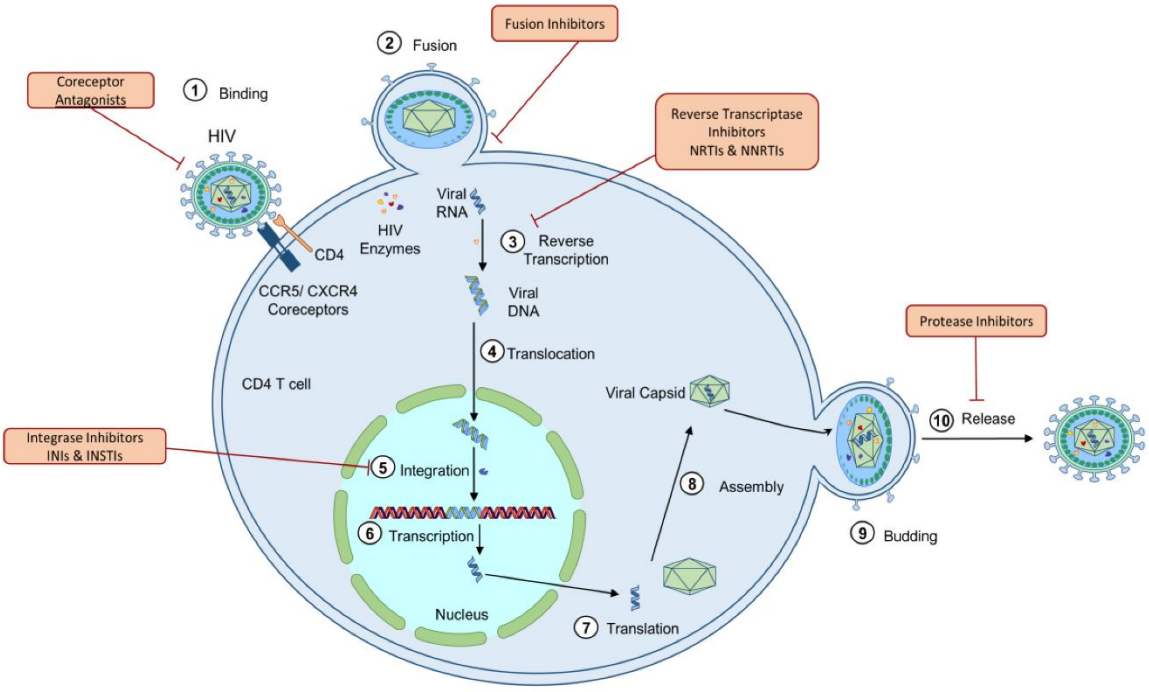
Appendix VIII

Invitrogen by Thermo-fisher scientific machine

Source:Author's Field work, 2022

Appendix IX

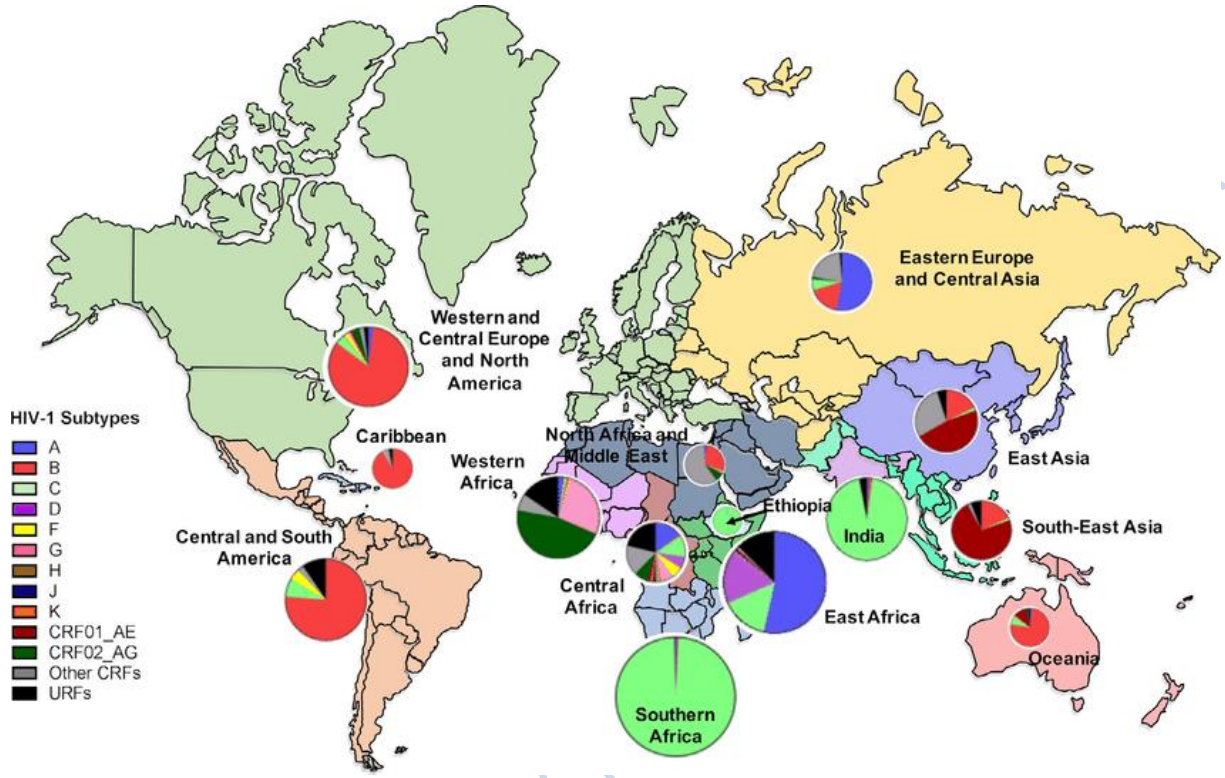
HIV Lifecycle



Source: Nature Medicine, 2010

APPENDIX X

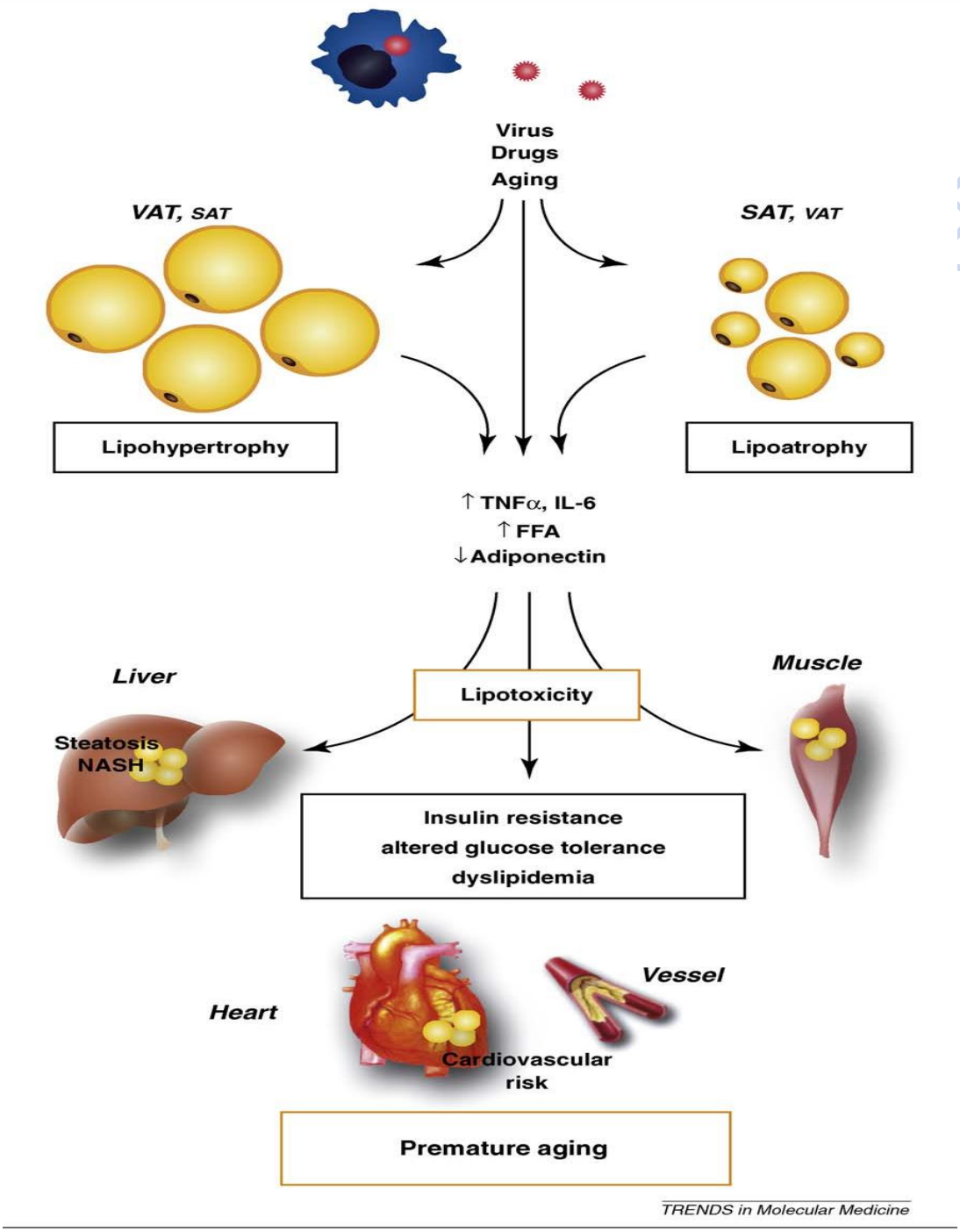
World Map Illustrating the Prevalence Of HIV-1-Group M-Subtypes Within Each Region Pie



Source: Research Gate, 2019

APPENDIX X

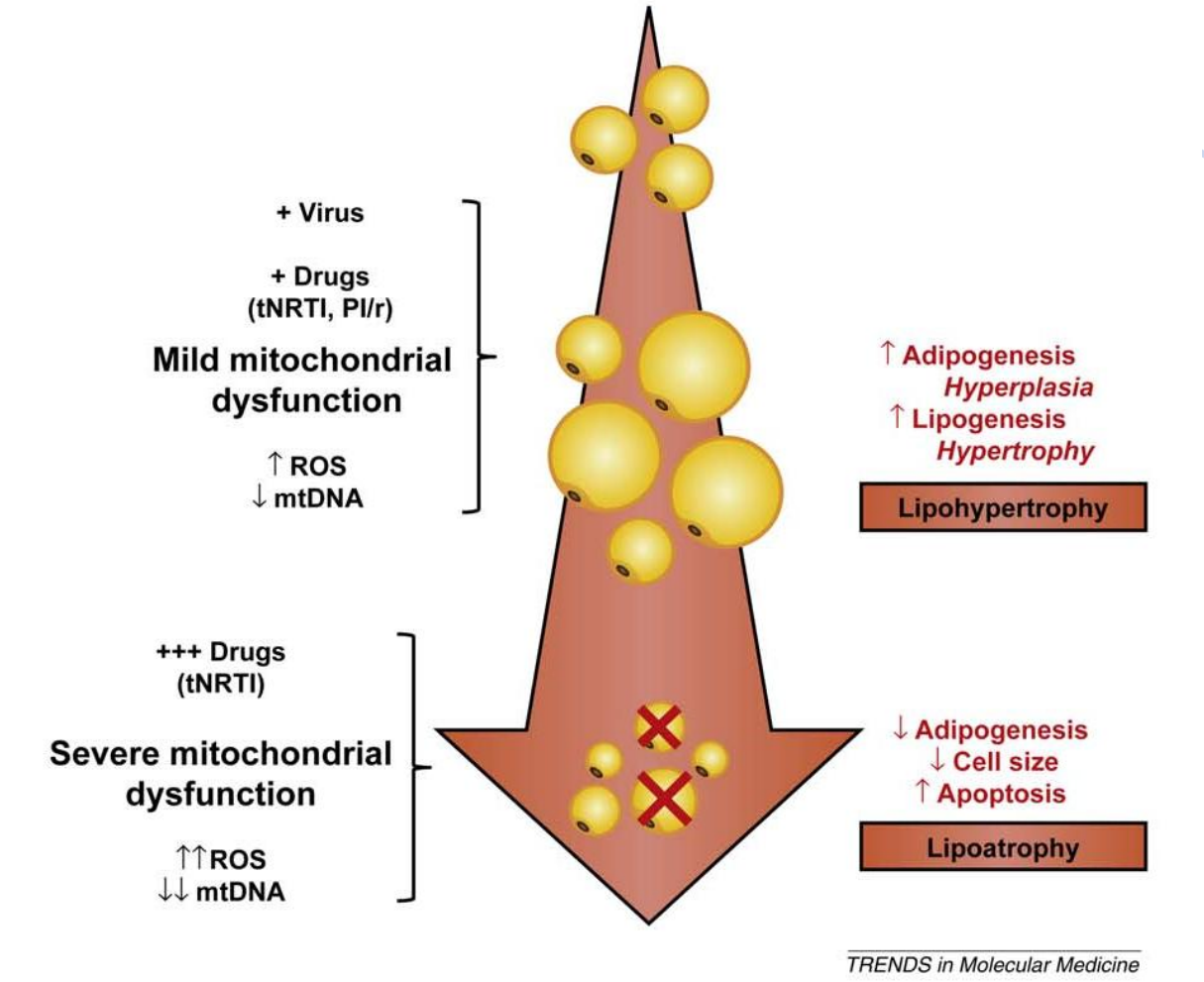
Illustrations of Organs Affected by HIV and Drugs



Source: Trends in Molecular Medicine,2020

APPENDIX XII

Illustration of Diseases/Dysfunction caused by HIV and ART



Source: Trends in Molecular Medicine, 2021

Informed Consent

Title Of Research:

Assessment Of Mitochondrial DNA Damage and Aneuploidy Among HIV-Positive Teenagers in Southwest Nigeria.

Principle Investigator, Affiliation and Contact Information:

Kordinum ALUMONA, Lead City University, Ibadan, (kordinum@gmail.com)

Supervisor and Affiliations:

Dr. C.K. Onwuamah,

Nigerian Institute of Medical Researchers (NIMR), Lagos

Institutional Contact: Biological Science Department,

Post Graduate School,

Lead City University.

08153318763.

Introduction and Purpose of The Study

Kordinum Alumona is a post graduate master's student at Lead City University, is conducting a research study to contribute to knowledge about the adverse effect of the new antiretroviral drugs being administrated to HIV positive teenagers within the age range of 13years to 19years in the south west geopolitical zone of Nigeria and how it affects the mitochondria DNA and the possibility of genome instability in teenagers receiving antiretroviral medications. She is asking for your consent to participate in this study, there would be no penalty if you choose to not participate, as you would receive the standard care available in this facility.

Description of The Research

When you agree to participate in this research study, Blood samples will be collected from both HIV positive teenagers receiving antiretroviral treatment and HIV negative teenagers, Basic information will be collected from the patient, including name, age, gender, height, and weight. This study is meant to cover the gap in knowledge with various laboratory analyses to be carried out in order to evaluate mitochondria DNA damage and aneuploidy in samples collected from both HIV-positive teenagers on antiretroviral medications in comparison to HIV-negative teenagers. It is a comparative study being carried out to know if there is the possibility of a large scale of exposure to unseen damages in the mitochondria DNA and it will also describe how this mitochondria DNA damage cause harm to an individual's healthcare. This study will be carried out for a period of 6months.

Subject Participation

We estimate that 60 participants who agree to participate in this study should be 30 HIV positive teenagers receiving therapy and 30 HIV negative teenagers in southwest geopolitical zone of Nigeria, between the age 13 – 19 years who can verbally communicate. Your participation will involve one visit approximately 5 minutes in length to enable sample and data collection.

Potential Risks and Discomforts

During sample collection, participants will feel a slight prick as sample is being collected, aside this, participants will not be exposed to any known risk.

Confidentiality

All information taken from the study will be coded to protect each participant's name. No names or other identifying information will be used when discussing or reporting data. The investigator(s) will safely keep all files and data collected in a secured locked cabinet in the Supervising investigator's office.

Authorization

By signing this form, you authorize the use and disclosure of the following information obtained from your sample, use of your records, any observation and findings found during this study for education, publication, and presentation of study findings.

Voluntary Participation and Authorization

As a participant, it is not mandatory to participate in this study, participation is completely voluntary. If you decide to not participate in this study, it will not affect the care and services which you're entitled to in this facility. There will be no interference with the current standard of care of patient.

Withdrawal From The Study and/or Withdrawal Of Authorization

If you decide not to continue participating in this study, you may withdraw at any time without penalty, and your blood samples will be disposed properly and none of your data will be collected or used.

COST: There is no cost for participating in this study. No extra medical expenses resulting from participation in this study.

Will You Be Compensated For Participating In This Study? There are no monetary benefits or compensation beyond the free provision of additional testing.

Who Can You Call If You Have Questions? If you or you should have any questions about this study, please feel free to contact the principal investigator, Kordinum ALUMONA (kordinum@gmail.com), Nigerian Institute of Medical Researchers IRB (nimr_irb@yahoo.com) 08141720523

I voluntarily agree to participate in this research program

Yes

No

I have read and understood this informed consent document and the information has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to participate.

I understand that I will be given a copy of this signed Consent Form.

Name of Participant:

Signature: **Date:**

Name of Witness:

Signature: **Date:**

Name of Research team member:

Signature: **Date:**

Note: A copy of the signed, dated consent form must be kept by the Principal Investigator(s) and a copy must be given to the participant.

I voluntarily agree to participate in this research program DUPLICATE

Yes

No

Name of participant: _____

Signature: -. **Date: -**

Name of witness: - _____

Signature: -. **Date: -**

Name of Research team member: _____

Signature: -. **Date: -**

I have read and understood this informed consent document and the information has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to participate.

Appendix Table I

Paticipant Data Form

Name *(*Positive Patients Only*)*
Code *(*Negative Patients Only*)*
Age
Sex
Height
Weight
Status
Location
Smoking
Drinking
Antiretroviral Treatment Administered At Time Of Sample Collection
Duration Of Antiretroviral Treatment
Types Of Antiretroviral Treatment Taken Over Time
Occupation
Signature

Source:Author's Field work, 2022

Appendix Table II

Positive Participant Data

Sample Id	Age (Yrs)	Gender	HIV Status	Location	Art	Duration
Nm-20-0007	18	M	Positive	Lagos	Tld	30 Months
Nm-16-0099	22	M	Positive	Lagos	Tld	75 Months
Nm-22-0026	16	F	Positive	Lagos	Atv/R/Abc/3tc	6months
Nm-20-0099	15	M	Positive	Lagos	Atv/R/Abc/3tc	22 Months
Nm-20-0148	15	M	Positive	Lagos	Tld	19 Months
Nm-16-0400	21	F	Positive	Lagos	Tld	42 Months
Nm-20-0124	15	M	Positive	Lagos	Atv/R/Abc/3tc	20 Months
Nm-20-0098	18	M	Positive	Lagos	Dtg/Abc/3tc	22 Months
Nmp-12-0015	15	M	Positive	Lagos	Tld	41 Months
Nm-18-0017	19	M	Positive	Lagos	Atv/R/Abc/3tc	39 Months
Nmp-13-0006	15	F	Positive	Lagos	Tld	126 Months
Nmp-12-0049	11	F	Positive	Lagos	Lpv/R/Azt/3tc	119 Months
Nm-18-0029	20	M	Positive	Lagos	Tld	43 Months
Nmp-09-0030	14	F	Positive	Lagos	Tld	160 Months
Nm-15-0502	22	M	Positive	Lagos	Tld	39 Months
Nm-22-0024	16	M	Positive	Lagos	Tld	5 Months
Nmp-17-0001	14	F	Positive	Lagos	Abc/3tc/Dtg	66 Months
Nmp-18-0008	9	M	Positive	Lagos	Abc/3tc/Dtg	45 Months
Nmp-08-0115	14	M	Positive	Lagos	Atv/R/Abc/3tc	62 Months
Nmp-11-0030	12	M	Positive	Lagos	Tld	110 Months

Nm-21-0215	16	M	Positive	Lagos	Tld	
Nmp-08-0062	14	M	Positive	Lagos	Abc/3tc/Dtg	168 Months
Nmp-19-0009	13	M	Positive	Lagos	Tld	36 Months
Nmp-11-0086	14	M	Positive	Lagos	Tld	131 Months
Nm-21-0080	20	M	Positive	Lagos	Tld	14 Months
Nmp-13-0041	12	F	Positive	Lagos	Tld	111 Months
Nmp-15-0045	18	F	Positive	Lagos	Azt/3tc/Efv	199 Months
Nm-21-0019	17	F	Positive	Lagos	Tld	15 Months
Nmp-14-0037	13	F	Positive	Lagos	Tld	95 Months
Nmp-07-0008	18	F	Positive	Lagos		178 Months

Source:Author's Field work, 2022

Appendix Table III

Negative Participant Data

001	13	F		29	Negative	Odk Str. Alakia, Ibadan
002	18	M	1.67	41	Negative	Alakia, Ibadan
003	19	M	1.70	42	Negative	Olodo, Ibadan
004	13	M	1.62	52	Negative	Ologuneru, Ibadan
005	14	M	1.57	42	Negative	Alakia, Ibadan
006	19	M	1.57	40	Negative	Olunde, Ibadan
007	15	F	1.42	38	Negative	Ibadan
008	19	M	1.58	43	Negative	Muslim Area, Ibadan
009	19	M	1.68	55	Negative	Muslim Area, Ibadan
010	19	F	1.40	41	Negative	Ibadan
011	14	M	1.79	68	Negative	Ologuneru, Ibadan
012	18	F	1.51	54	Negative	Ibadan
013	19	F	1.50	52	Negative	Ibadan
014	19	M	1.72	62	Negative	Uch, Ibadan
015	14	F	1.62	45	Negative	Oluwo Area, Ibadan
016	16	M	1.66	55	Negative	New Garage, Ibadan
017	14	M	1.42	32	Negative	Ibadan
018	15	F	1.29	34	Negative	Mokola, Ibadan
019	14	M	1.35	32	Negative	Iwo
020	14	M	1.58	35	Negative	Olorunsogo, Ibadan
021	14	M	1.67	40	Negative	Akanrami, Ibadan
022	19	F	1.50	50	Negative	Agodi-Gate, Ibadan
023	19	F	1.52	48	Negative	Sawmill, Ibadam
024	16	F	1.58	42	Negative	Bodija, Ibadan
025	13	M	1.19	25	Negative	Iwo Road, Ibadan
026	16	F	1.48	29	Negative	Ibadan
027	17	F	1.51	32	Negative	Monatan, Ibadan

028	18	F	157	48	Negative	Agodi-Gate, Ibadan
029	13	F	1.25	29	Negative	Muslim Area, Ibadan
030	15	F	1.60	42	Negative	Idi-Agbon, Soka, Ibadan

Source:Author's Field work, 2022

Appendix Table IV

Results of triplicate analysis

Sample	ND1	ND4	B2M	Δ Ct	Del %	Copy # %
C01a	21.09	18.96	23.95	2.13	22.04	88.05846
C01b	21.14	18.96	23.73	2.18	21.04	89.08555
C01c	21.01	18.84	23.97	2.17	21.24	87.65123
C03a	21.3	19.08	24.02	2.22	20.29	88.6761
C03b	21.31	19.15	24.23	2.16	21.43	87.94882
C03c	21.41	19.12	24.18	2.29	19.07	88.54425
C04a	20.99	18.96	23.03	2.03	24.27	91.14199
C04b	20.94	18.84	23.13	2.1	22.68	90.53178
C04c	20.98	18.92	23.04	2.06	23.56	91.05903
C05a	21.43	19.15	24.12	2.28	19.24	88.84743
C05b	21.43	19.19	24.23	2.24	19.93	88.44408
C05c	21.49	19.24	24.24	2.25	19.75	88.65512
C06a	21.64	19.54	24.8	2.1	22.68	87.25806
C06b	21.61	19.49	24.67	2.12	22.25	87.59627
C06c	21.58	19.59	24.59	1.99	25.25	87.75925

C07a	20.22	18.32	23.34	1.9	27.70	86.63239
C07b	20.27	18.29	23.14	1.98	25.51	87.59723
C07c	20.22	18.28	23.14	1.94	26.57	87.38116
C08a	21.23	19.07	24.17	2.16	21.43	87.83616
C08b	21.25	19.2	24.23	2.05	23.80	87.7012
C08c	21.21	19.2	24.39	2.01	24.75	86.96187
C09a	20.12	17.85	22.61	2.27	19.41	88.98717
C09b	20.06	17.93	22.69	2.13	22.04	88.40899
C09c	19.88	17.87	22.7	2.01	24.75	87.57709
C10a	20.7	18.59	23.36	2.11	22.46	88.61301
C10b	20.73	18.8	23.5	1.93	26.85	88.21277
C10c	20.7	18.82	23.5	1.88	28.29	88.08511
C11a	21.54	19.78	24.28	1.76	32.28	88.71499
C11b	21.36	19.54	24.33	1.82	30.19	87.79285
C11c	21.43	19.65	24.22	1.78	31.56	88.48059
C12a	20.04	18.15	22.79	1.89	27.99	87.9333
C12b	19.95	18.01	22.89	1.94	26.57	87.15596
C12c	20.06	18.13	22.8	1.93	26.85	87.98246
C17a	21.12	19.22	24.35	1.9	27.70	86.73511
C17b	21.13	19.22	24.38	1.91	27.41	86.6694
C17c	21.11	19.26	24.41	1.85	29.22	86.48095
C18a	20.29	18.35	24.19	1.94	26.57	83.87764
C18b	20.27	18.41	24.23	1.86	28.91	83.65662

C18c	20.22	18.34	24.24	1.88	28.29	83.41584
C19a	20.53	18.73	23.39	1.8	30.86	87.77255
C19b	20.53	18.59	23.34	1.94	26.57	87.96058
C19c	20.64	18.69	23.18	1.95	26.30	89.04228
C20a	21.28	19.38	23.83	1.9	27.70	89.2992
C20b	21.3	19.41	23.85	1.89	27.99	89.30818
C20c	21.24	19.36	23.94	1.88	28.29	88.7218

Source:Author's Field work, 2022

Appendix Table V

Results of triplicate analysis

S16a	18.77	18.6	23.88	0.17	3460.21	78.60134
S16b	18.79	18.62	24.45	0.17	3460.21	76.85072
S16c	18.99	18.93	24.17	0.06	27777.78	78.56847
S17a	18.04	18.78	23.96	-0.74	182.62	75.29215
S17b	18.08	18.73	24.06	-0.65	236.69	75.14547
S17c	18.08	18.91	23.9	-0.83	145.16	75.64854
S18a	15.83	16.79	20.59	-0.96	108.51	76.88198
s18b	16.01	16.51	20.7	-0.5	400.00	77.343
s18c	16.06	16.74	20.76	-0.68	216.26	77.36031
s19a	17.4	18.25	23.24	-0.85	138.41	74.87091
s19b	17.45	18.28	23.22	-0.83	145.16	75.15073
s19c	17.52	18.27	23.32	-0.75	177.78	75.12864
s20a	16.08	16.96	22.55	-0.88	129.13	71.3082
s20b	16.3	17.4	22.39	-1.1	82.64	72.80036
S20c	16.2	16.96	22.91	-0.76	173.13	70.71148
S21a	17.94	17.87	22.97	0.07	20408.16	78.10187
S21b	17.98	17.84	22.95	0.14	5102.04	78.34423
S21c	17.7	17.74	22.95	-0.04	62500.00	77.12418
S22a	17.13	17.83	22.3	-0.7	204.08	76.81614
S22b	16.6	17.4	22.39	-0.8	156.25	74.14024
S22c	16.64	17.53	22.41	-0.89	126.25	74.25257

S23a	17.3	18.11	23.64	-0.81	152.42	73.18105
S23b	17.05	17.98	23.52	-0.93	115.62	72.4915
S23c	17.23	17.97	23.5	-0.74	182.62	73.31915
S24a	17.79	18.6	23.92	-0.81	152.42	74.37291
S24b	17.85	18.59	23.85	-0.74	182.62	74.84277
S24c	17.79	18.71	23.8	-0.92	118.15	74.7479
S25a	17.77	18.71	23.65	-0.94	113.17	75.13742
S25b	17.69	18.56	23.62	-0.87	132.12	74.89416
S25c	17.71	18.42	23.5	-0.71	198.37	75.3617
S26a	17.77	18.64	23.7	-0.87	132.12	74.9789
S26b	18.14	18.86	23.79	-0.72	192.90	76.25053
S26c	17.66	18.48	23.9	-0.82	148.72	73.89121
S27a	17.34	18.05	23.06	-0.71	198.37	75.19514
S27b	17.51	18.22	23.97	-0.71	198.37	73.04965
S27c	17.31	18.02	22.84	-0.71	198.37	75.78809
S28a	16.88	17.69	22.41	-0.81	152.42	75.32352
S28b	16.78	17.61	22.5	-0.83	145.16	74.57778
S28c	16.95	17.73	22.68	-0.78	164.37	74.73545
S29a	16.87	17.67	23.72	-0.8	156.25	71.12142
S29b	18.57	19.27	22.67	-0.7	204.08	81.91442
S29c	18.48	19.24	22.87	-0.76	173.13	80.80455
S30a	16.53	17.29	22.01	-0.76	173.13	75.10223
S30b	16.43	17.23	22.08	-0.8	156.25	74.41123

S30c	16.31	17.16	21.98	-0.85	138.41	74.20382

Source:Author's Fieldwork, 2022

Bio-Data

A. Personal Data

Full Name: ALUMONA Kordinum

Address: No 15, Lane 5, Abidiogun Estate, Adeoyo Ring-road, Ibadan, Oyo State.

Email Address:Kordinum@gmail.com

Phone Number: 08027219813, 08143145041

Date of Birth: 6th August 1998

Place of Birth: Warri, Delta State, Nigeria.

Nationality: Nigerian

Next of Kin: Alumona Ekenedinichukwu

Address of Next of kin: 16, Dumebi Amaka Isidi Street, off DLA road, Asaba, Delta State.

B. Educational Background

I. **Primary Education: -** Classical International School, Off PTI road, Warri, Delta State (2002-2010)
First School Leaving Certificate

II. **Secondary Education: -** Hollywood Bilingual International School, Asaba, Delta state (2010-2013)
Senior School Leaving Certificate Examination (WAEC and NECO)

III. **First degree: -** Bachelor of Science in Medical Biochemistry and Genetics
Delta State University, Abraka, Delta State (2014-2015)

IV. **Second degree: -** Master of Science in Molecular Biology and Genomics
Lead City University Ibadan, Oyo State (2020-2022)

C. Working Experience

Laboratory Assistant
Ring-road State Hospital, Adeoyo, Ring-road, Ibadan, Oyo State (2020)

Laboratory Assistant (INTERN)
Emmanuel Laboratory Center, Police Station Road, Abraka, Delta State (2017)

D. Awards and Fellowships

1. Award for Best Oral Abstract at the International Conference on Health Advances, Innovation and Research, at Nigerian Institute of Medical Research (NIMR).

2. The Dangote Foundation Scholar for the International Conference on Health Advances, Innovation and Research, at Nigerian Institute of Medical Research (NIMR).

E. Publications

Major conferences / Workshops

1. Oral Abstract presentation on the at the International Conference on Health Advances, Innovation and Research (ICHAIR,2022), at Nigerian Institute of Medical Research (NIMR).

2. Postal abstract presentation at the 3rd International Conference Faculty of Natural and Applied Sciences (FASCON 2022), at Lead City University.

3. IBMT Vaccine Design Workshop (2022)

4. Bioinformatics and Molecular Biology Hands on Training on Pathogens and Cancer Research Workshop 2021

…… …….

Signature **Date**

University Compliance Certification

This is to certify that the thesis by **Alumona Kordinum** with Matric no **LCU/PG/001932** in the Department of **Biological Science**, Faculty of Natural and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

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Signature **Date**

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