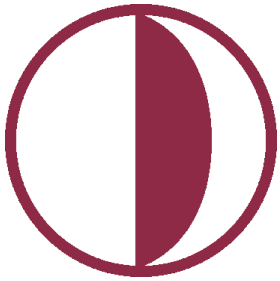

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NEAR EAST UNIVERSITY
HEALTH SCIENCES INSTITUTE

ANTIVIRAL DRUG RESISTANCE IN ADULT PATIENTS INFECTED WITH HIV-1

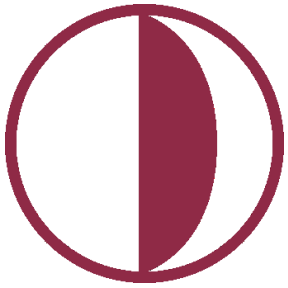
AHMED SHARIF

Masters Thesis

MEDICAL MICROBIOLOGY
AND CLINICAL MICROBIOLOGY DEPARTMENT

MENTOR
PROF. DR. MURAT SAYAN

2019-NICOSIA



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This study has been accepted by the thesis committee of medical microbiology and clinical microbiology programme as Master Thesis.

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Approval

According to the relevant articles of Near East University postgraduate study education and examination regulations, this thesis has been approved by the above mentioned members of the thesis committee and the decision by the board of directorate of the institute.

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ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral drugs
HIV	Human Immunodeficiency Virus
CDC	Centre for Disease Control
HAART	Highly Active Antiretroviral Therapy
LTNP	Long-term Non-Progress
MENA	Middle East and North Africa
MSM	Men who have sex with men

ÖZET

HIV salgını hala başlıca küresel ölüm sebebidir, bu küresel sağlık tehditinin henüz aşısı yok. Antiretroviral ilaçlar bu büyük katilin tedavisine umut ışığı getirdi. Ancak HIV hastasına karşı olan etkileri direnç gelişmesi sebebiyle azaltıldı. Antiretroviral ilaç direncinin bu ürkütücü gelişimi temel olarak direnç genlerinin dünya genelinde hızlı bir şekilde oluşumu ve yayılması üzerinedir. Bundan dolayı bu araştırmanın amacı subtip oluşumunun doğasını ve türkiyede tedavi görmemiş hastalardan izole edilen ilaç dirençli HIV-1 suşlarının mutasyonunu belirlemektir. Toplamda 103 yeni tanımlanmış HIV-1 pozitif hastası çalışmaya dâhil edildi. HIV-1 subtiplerini tespit etmek için genetik metot kullanıldı, dolaşımdaki rekombinant form (CRF) ve ayrıca ilaç direnci mutasyonları 2009 Dünya sağlık kuruluşunun ilaca dirençli mutasyonların izlem listesine göre analiz edildi. Sonuçlar şu kategorileri ortaya koydu; subtip B, non subtip B ve CRF. Sonuçlara göre, subtip B yüzde 58 (%56.31) ile baskın, non subtip B yüzde 34 (%33.0) ve CRF yüzde 11 (%10.67) sini oluşturuyor. NRTI, NNRTI ve PI için HIV-1 ilaç direnci oluşumları sırasıyla %7.76 (103 hastadan 8si), %22.3 (103 hastadan 23si) ve %0.97 (103 hastadan 1i) dir ve hastalar NRTI için şu primer antiretroviral ilaç direnci mutosyonları sahiptir; 3 A62V (% 2.91), 2 M41L (% 1.94), 1 F77L (%0.97), 1 K70KR (%0.97) ve 1 T215S (%0.97) ilaç direnci mutasyonu bulundu. NNRTI ilacında 13 E138A (%12.62), 3 E138G (%2.91), 3 K103N (%2.91), 2 V179D (%1.94), 1 K103KN (%0.97) ve 1 K103T (%0.97) ilaç direnci mutasyonu bulundu. PI ilacında 1 L24LI (%0.97) ilaç direnci mutasyonu bulundu. Bu araştırma antiretroviral terapi denenmemiş hastalarda primer ilaç direncinin oluşumunu doğrulamış ve direnç tesbitinin; hangi sırayla uygun olan birinci ilaç seçimine rehberlik edecek HIV tedavi planında gerekli olduğunu önermiştir.

ABSTRACT

Human Immunodeficiency Virus pandemic is still leading cause of dead globally, this global threat to global health has no vaccine yet. Antiretroviral drugs brought ray of hope in the treatment of this major killer; however, their effectiveness against HIV patients is reduced due to the development of resistance. The daunting growth of antiretroviral drug resistance is mainly on the rapid occurrence and spread of the resistance genes across the globe. Hence, the aim of the research is to determine the nature of subtype occurrence and type of mutation of drug-resistant HIV-1 strains isolated from patients who are not exposed treatment in Turkey. A sum of 103 newly identified HIV-1 positive patients were recruited into the study. Genetic method was employed to detect HIV-1 subtypes, circulating recombinant forms (CRFs) and also drug resistant mutations were analysed according to the 2009 World Health Organization list of monitoring drug-resistant mutations. The results revealed the following categories; Subtype B, non-subtype B, and CRF. According to the results, subtype B was dominant 58 (56.31%), non-subtype B had 34 (33.0%) and CRF had 11 (10.67%). The occurrence HIV-1 drug resistance for NRTIs, NNRTIs, and PIs were 7.76% (8/103), 22.3% (23/103) and 0.97% (1/103) respectively and the patients had the following primary antiretroviral drug resistance mutations to NRTI, 3 A62V (2.91%), 2 M41L (1.94%), 1 F77L (0.97%), 1 K70KR (0.97%), and 1 T215S (0.97%) drug resistance mutations were found. In the NNRTI, 13 E138A (12.62%), 3 E138G (2.91%), 3 K103N (2.91%), 2 V179D (1.94), 1 K103KN (0.97%) and 1K103T (0.97%) mutations were found . In the PI drug, 1 L24LI (0.97%) drug resistance mutations was found. This research confirms the occurrence of primary drug-resistant mutations in ART-naive patients and recommends that resistance detection is suggested to be included in the HIV treatment plan, which in turn will guide the choice of appropriate first-line drug regimen.

Keywords: HIV, resistance, naïve patients, antiretroviral agents, mutations.

1.1. INTRODUCTION

This chapter gives the overall introduction to the HIV infection; its types, HIV lifecycle, the aim of the study and objectives, as well as significance of the study.

The human immunodeficiency virus (HIV) remains one of the top leading causes for mortality worldwide. Historically, the virus was first discovered in the early 80s among men having sex with men in USA who complained with *Pneumocytiscarinii pneumonia* and currently it is estimated about 3.7 million individuals living with HIV/AIDS infection at the end of year 2015 (Jacobs et al., 2008; WHO, 2016). The mortality from the infection is increasing especially in developing countries enveloped by many factors such as lack of early diagnosis, lack of education or lack of antiretroviral drugs. The HIV is a member of retrovirus family, genera *Lentivirus* with some common features of ribonucleic acid (RNA) viruses including high rate mutation, small genome and similar number of offspring (Nguyan, 2012). HIV infection continually keep spread in Sub-Saharan African and approximately about 2 million individuals were infected in 2009 and the data of those living with HIV in the region is reported to have around 22.5 million in the region. Conversely, in Europe the HIV-1 epidemic is lesser than other regions of the world with an estimated 130,000 newly diagnosed in Western and Central Europe in the same year (Pingen et al., 2011). The uncertain pattern of the disease in different part of the world would be based on some explanations such use of antiretroviral drugs, reduction in risk sex in certain part of the world and screening of individuals.

Pingen et al., (2011) extensively traced the origin of HIV to people in Democratic republican of Congo that are tested in 1959. In addition, molecular and phylogenetic studies estimated the introduction of HIV into human population as back as 1930s. The relatedness of HIV to Simian Immunodeficiency Viruses (SIVs) that is isolated in non-human primates, a zoonotic disease adapted to human host (Korberet al., 2000). The authors describe the degree of infectivity between the two related virus families, where SIVs do not cause great impact on non-human counterpart as HIV in human primates. Other studies have tremendously look at the origin of HIV and its closely related sibling; SIV in wild Chimpanzees indicating similarity in AIDS-like disease condition (Keele et al., 2009; Nguyan, 2012). This account for the evolutionary changes and

adaptation between close related infection in nature. Thus, HIV exists as a repository of many genetically distinct strains of viruses (quasi-species).

1.2. Types Of HIV

There exist two genetically distinct types of HIV retrovirus and principally they differ in nature of infectivity and geographical distribution. Campbell-Yusufu and Ghandi (2011) state that HIV virus exists in two forms; HIV type 1and HIV type 2, and epidemiologically the HIV-1 is the major cause of global AIDS pandemic while HIV-2 is account for diseases in West African countries such as Cameroon, Mali, Nigeria, Sierra Leanne, Cote d`Ivoire, Cape Verde and Senegal. The work of Luc Montagnier and his associates at the Institute Pasteur in Paris in1983 led to discovery of HIV-1 while HIV-2 was first discovered in Senegal in 1983.

In addition, the HIV Type1variants are further grouped into four (4) main categories: group M (main), group N (non-M/non-O), group O (outlier) and group P. The group that cause large infection globally is mainly Group M (Klimas et al., 2008). The HIV-1 is further classified into 10 more subtypes which are tagged as subtype A-K (Fig. 1.); this indicated the rapid evolutionary dynamics of the virus. In addition, the authors stated that the HIV-1 subtypes A and C as the main cause of HIV infection pandemic, among the subtypes also there is variability of location, for instance HIV-1 subtype B is considered predominantly in Australia, North America and Western Europe. Sarrs et al., (2000) have described the phylogenetic analysis of nucleotide sequences of HIV-type 1 as the basis of the classification of the subtype.

During infectivity, HIV-1 specially binds to cells having CD4a glycoprotein which has a part in immune process. The process of binding occurs through specific interactions between the viral envelope GP120 and the amino-terminal end of immunoglobulin unit of CD4. However, it still requires more cell-surface proteins to facilitate binding of the viral and cellular surfaces. This interaction can trigger many chemokine receptors, including CXCR4 and CCR5 (Turner and Summers, 1999).

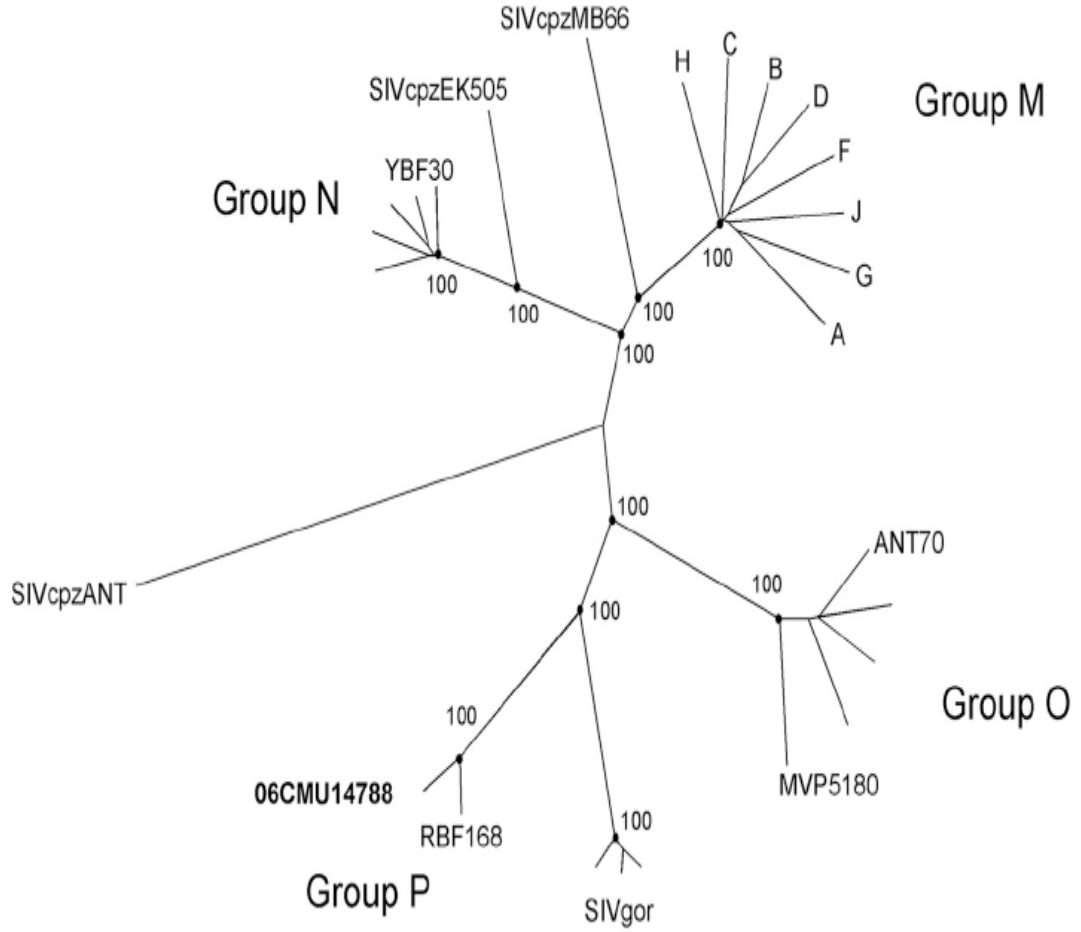

Figure 1.1 HIV-1 Phylogenetic tree derived from nucleotide alignment of genome sequences. (Jacobs *et al*., 2011).

HIV Type 2 variants have five distinct and roughly equidistant evolutionary lineages by analogy with HIV-1, but less little diversity with only two subtypes A and B in contrast to HIV-1 (Sarr et al., 2000). Other reports indicate that there exist four other subtypes (C-F) but the isolation on new infected individuals met setback (Akimoto et al., 1998; Jaafar et al., 2004). Jaafar et al., (2004) in an extensive review on HIV-2 distribution indicated Sub-Saharan African to have over 10% prevalence that other part of the world. A considerable amount of literature has been published both agreed that HIV-1 and HIV-2 have evolved from same progeny and presents same disease conditions with main difference in epidemiological distribution across the world.

1.3. HIV Life Cycle

It has been demonstrated that HIV lifecycle as one that exhibit classical viral lifecycle with wide range of stages. Kirchoff (2013) gave an overview of HIV life cycle in a comprehensive review and termed it "replication" which involved several steps that allow changes to the virus when first exposure to the target cell which results to the production of new infectious viral units and subsequent infection of next host. These small infectious agents of diseases in human sized 20 to 300 nm and unlike other microbes live in the host cell for survival, thus totally dependent on the host for replication (Abbas, 2000). HIV consists of an outer envelope covered with lipid bilayer accompanied with extensions of glycoproteins (GP); GP 41 and 120 which are connected to allow GP 120 bulges the surface of the virus. This complex structure contains arrangement of nucleocapsid around the central core of protein.

Two copies of single-stranded RNA are located in the core which serves as the virus genome which regulate the gene expression of the virus. Many molecules of enzymes, reverse transcriptase (RT) are located within the core and result to converting viral RNA into provial DNA (Abbas, 2000). HIV has specificity to certain cells that have CD4 receptors in human body. Kirchhoff (2013) described the various HIV replication cycle; Attachment, binding and fusion, reverse transcription, uncoating and nuclear entry, integration, transcription, translation and assembly, budding, maturation and final release of complete viral particle (Fig.1.2).

The Viral attachment: HIV that have not been attached to host has about 20-30 minutes half-life in infected person, thus necessitate attachment to the new host within short time. As described by many studies, the main receptor is CD4 and secondary receptors which are known as co-receptors, chemokine receptors CCR5 and CXCR4 which allow viral entry, thus prone cell susceptible to HIV entry (Jaffar et al.,2004; Abbas, 2000; Kirchhoff, 2013). Similarly, other receptors have been described to be attachment cell for HIV virus in an unspecified pattern, such receptors include poly-glycans, lectins, and others.

Another important step of HIV replication cycle is binding and fusion to the host cell which initiate infection process by the interplay with external viral glycoproteins

gp120 with cellular CD4 receptors which result to conformational changes in the viral particle. Once this is achieved, the genetic information of the virus enters, and initiates process of genetic takeover of the host cell. The HIV sum of the genes is consisted of two stranded RNAs that are covered by nucleocapsid. After subsequent binding, leads to the transcription process where the single stranded viral RNA is converted to double-stranded DNAs, contrary to normal transcription process of DNA to RNA by generation messenger RNA. It has been demonstrated that the uncoating of viral genetic constituent to the host cell as the important step in converting host genome.

The generated double-stranded DNA would allow gene expression and production of infection which can be achieved by enzyme, *integrase.* After complete process transcription and translation, matured virion particle will be bud and goes into circulation.

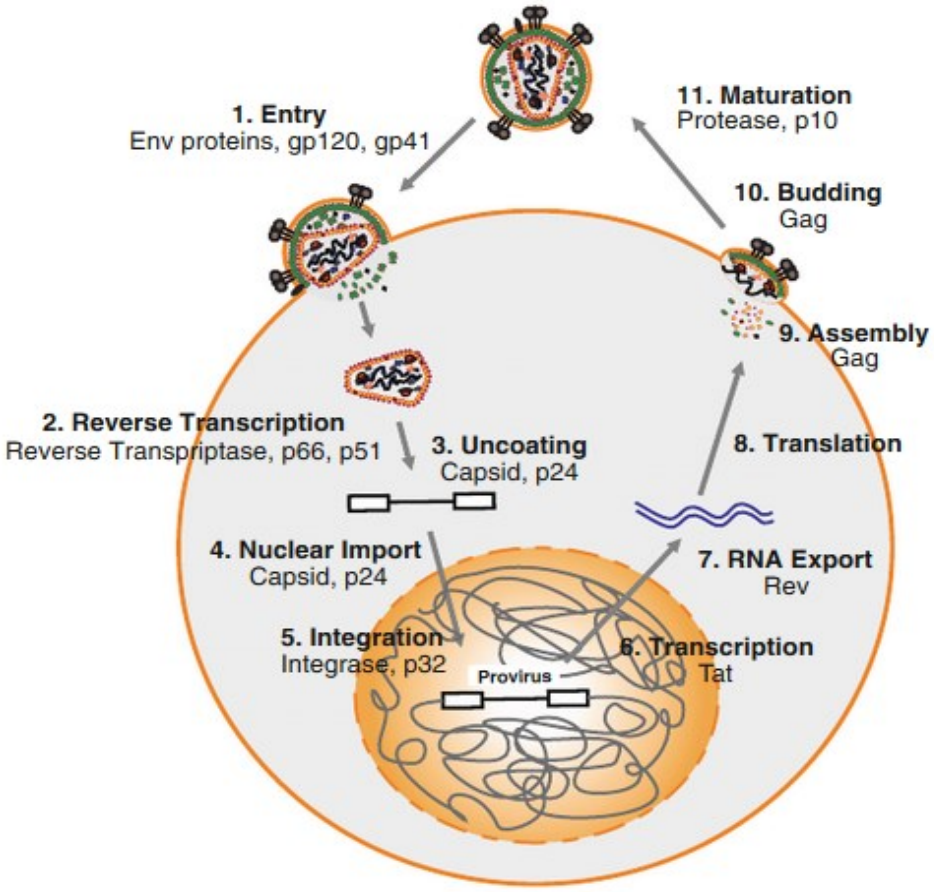

Figure 1.2. Overview of viral replication cycle. Adapted from (Kirchoff, 2013).

1.4. Significant Of The Study

This project will provide information of the nature of HIV-1 resistance profile amongst adult patients in Turkey and major driving factors contributing for the occurrence of resistance in the study area in comparison to other strains of HIV. This in turn will help the stakeholders with data of the resistance of HIV pattern in Turkey.

1.5. Aim and Objective Of The Study

- Identification of the occurrence of HIV-1 in Turkey and contributing factors the current HIV in the region
- To determine the resistance profile of HIV-1 isolate from Turkey.

2.1. GENERAL INFORMATION

2.1.1. HIV transmission

Globally, African region is considered high hotspot of HIV burden which account to 25.7 million at 2017 as representing over two-third of global HIV infections (WHO, 2018). The transmission risks are as follows; MSM, drugs abusers, inmates and other close association settings, prostitutes and their partners, and transgender persons.

The transmission of HIV is reported by many studies with different dimensions (Hollingsworth et al., 2008; Show and Hunter, 2012; Hoenigl et al., 2016). Shaw and Hunter (2012) extensively studied the transmission of HIV and attributed mainly to sexual contact across mucosal surfaces, by maternal-infant exposure, and by any contact with body fluids with damaged skin. Similarly, it is observed that sexual life styles play important role in the transmission of HIV as indicated by the studies of Hoenigl et al., (2016). The authors constructed a model to analyse the sequence of newly infected HIV case in South-East Austria in period of six years, interestingly the cohort study showed a high rate of inferred clustering within the cohort, the main people at risk are indicated to be MSM and injection drug abusers, and a number of international correlations. Furthermore, the study based on gender revealed that HIV positive amongst the males in the study area are identified as HSX transmission as the main chance of HIV infection for HIV, in comparison to other settings in Europe countries and the United States. The transmission in those areas concluded the existence of high risk of transmission in MSM in early HIV infections (Hoenigl et al., 2015; Hoenigl et al., 2016).

In Turkey, the transmissions mainly are linked to imported cases by foreign nationals, followed by drugs use and minimally by blood transfusion (Gülümser and Erbaydar, 2015). In the last decade, contrary to the world's decrease in HIV cases, Turkey faced dramatic increase by about 465% according Health ministry and all attributed to foreign importation by the neighbouring countries (Ersan, 2017). Turkey became receiving pool from the increase in its surrounding countries; the increase in Belarus, Ukraine and Russia to our north, eastern Europe to the west and Arab countries to the south was around 200 percent. Hence, contribute to the increase in

transmission in the country. Alarmingly, it is projected to have increase of over 39000 by the end of 2028 (Ersan, 2017). The general possible routes of transmission of HIV in Turkey are summarized in table (1.1). Recent report also indicated significant increase in HIV infection in Turkey in 2018, in a report by the Turkish ministry of health the number of individuals who are HIV positive in Turkey in 2018 reached 17.884, 1651 are with AIDS, 79.2% of total were males and 20.8% females and 15.2% were foreigners. In decreasing order, the transmission of HIV can be possible through the following means; unprotected sexual intercourse, injection drug abusers, infected blood transfusions, mother to child during birth or breastfeeding.

Table1.1. Distribution of possible transmission ways of HIV/AIDS cases in Turkey according to reporting period.

Possible ways of transmission	1985-1996		1997-2001		2002-2006		2007-2011	
	N	%	N	%	N	%	N	%
Heterosexual relationship	254	41.2	421	59.5	668	54.8	1410	52.6
Homosexual relationship	65	10.5	40	5.6	105	8.6	240	9.0
Intravenous drug use	71	11.5	26	3.7	25	2.1	33	1.2
Blood transfusion	44	7.1	6	0.8	4	0.3	14	0.5
Mother to child	6	1.0	12	1.7	25	2.1	27	1.0
Nosocomial	-	0.0	5	0.7	9	0.7	10	0.4
No data	177	28.7	198	28.0	383	31.4	946	35.3
Total	617	100.0	708	100.0	1219	100.0	2680	100.0

Adapted from (Ersan, 2017)

2.2. Antiretroviral Drugs

Viral lifecycle has been excellent targets for antiviral agents which can be selectively targeted with little effect to the host cell. Currently, there is no effective preventive vaccine but to reduce the viral load and boast immune system is the option to tame the infection using antiretroviral agents. In the past three decades, numerous studies have been put to quell the threat of viral infections using chemotherapeutic agents and mainly targeting intracellular mechanisms needed for the synthesis of viral biomolecules such as the proteins and nucleic acid, a number of agents are also employed to block assembly, maturation, budding, entry or uncoating act on virions, as shown in Figure (2.1.) (Bean,1992; Clercq, 2012; Menedez-Arias and Gago, 2013).

Despite the massive achievement in fighting against AIDS, few drugs have been approved, this challenge account by some factors such as; the nature of the virus which

have already developed substantive replication before appearing of clinical symptoms, obligatory intracellular parasitic nature that uses host machinery in each of its replication cycles, thus high selective is required to avoid the effect on host, development of resistance, and decrease in development of new agents. Each replication step is potential target for antiviral agents, but this work focuses on agents that are effective against HIV virus (Fig.3).

The studies to date have tended to focus on the main targets of anti-HIV drugs as follows (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs): with classical example such as zidovudine, didanosine, zalcitabine, lamivudine (ii) Another anti-HIV target is non-nucleoside reverse transcriptase inhibitors (NNRTIs) with the following examples delavirdine, emivirine, efavirenz, nevirapine (iii) Protease inhibitors (PIs) as a target has the following drugs as examples amprenavir, indinavir, lopinavir, saquinavir, ritonavir and nelfinavir. HIV replication cycles is considered an excellent targets to the agents, apart from reverse transcriptase (RT) and protease reaction, other metabolic activities can be employed during the replicative cycle as a promising targets for treatment plan: (i) The initial step, viral adsorption, that involves attaching to GP120 and negatively charged albumins (ii) Subsequent step is viral entry, can be use to block viral co-receptors CXCR4 and CCR5 (iii) The firm attachment requires virus–cell fusion, by attaching to GP41 (iv) After the synthesis of the viral molecules are assembled and spread, via NCp7 zinc finger-targeted agents azadicarbonamide (ADA); (v) proviral DNA integration, via integrase inhibitors (vi) viral mRNA transcription are all use as a promising point of anti-HIV agents (table 1) (De clercq, 2002; De clerq, 2009; Geronikaki et al., 2016).

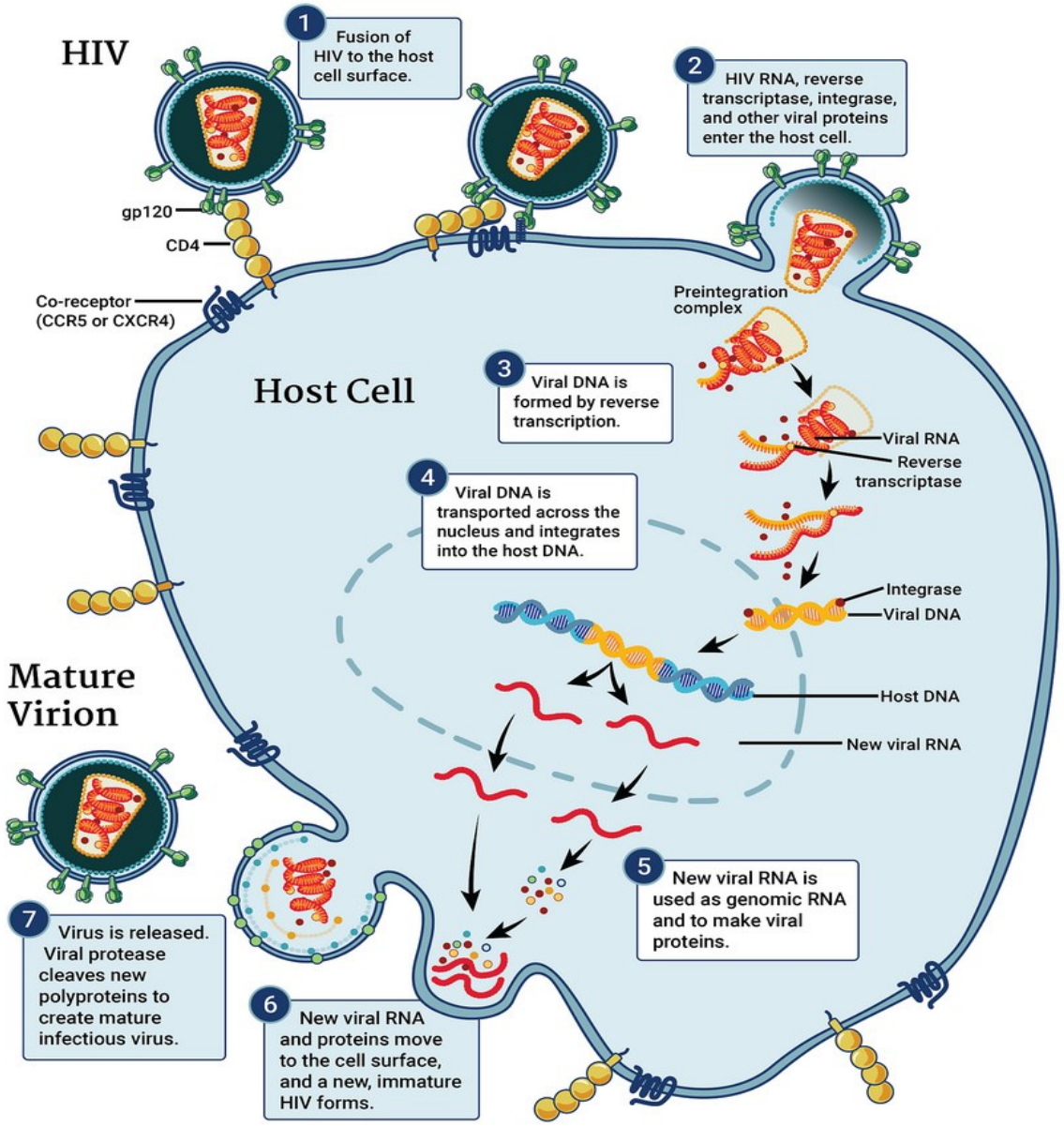

Figure 2.1. Replicative cycle of human immunodeficiency virus (HIV), highlighting the principal targets for therapeutic intervention: (co-) receptor interaction; virus–cell fusion; reverse transcription (by reverse transcriptase); integration; and proteolytic processing (by viral protease). Adapted from (De Clercq, 2009).

2.2.1. Nucleoside reverse transcriptase inhibitors (NRTIs)

This group of antiretroviral agents is among the first agents in treatment of HIV and has been extensively studied. This group contains seven members that are licensed

by Food and drug Administration (FDA), which involve abacavir, didanosine, emtricitabine, stavudine, tenofovir, lamivudine, zidovudine and zalcitabine (FDA, 2012). Structurally, they have been described as analogue of natural nucleoside and the all members contain similar backbone. The main mechanism of action of these drugs is to target unusual process of HIV replication which involves reverse transcription by taking advantage of the host cells enzymes, the dNTPs found in the human cell. When the new DNA of the virus is integrated into the human cells, chain termination occurs due to the lack of a 3'hydroxyl group in the NRTI (Coven, 2010). This important group can be used with different ART agents in combination to enhance activity and stop the occurrence of resistance. They are metabolized into their active triphosphate form prior to reverse transcriptase inhibition by the host kinases (Ravichandran et al., 2008). In their overview, the authors described Azidothymidine (AZT, Zidovudine) (Fig. 2.1) is the first FDA-approved NRTI in 1987. Other conjugate of AZT such as Fozivudinetidoxil and Tenofovirdisoproxilfumarate have been employed in the infection therapy. Among the members of this class Lamivudine has been described as the most potent agent though this activity is confounded with non-selectivity and rapid development of mutation with would fasten resistance. Conversely, Stavudine (d4T) is been described to have great antiviral activity in solo (Coven, 2010). Importantly, NRTIs have demonstrated significant activity in the treatment of the virus infection whether used in combination or alone.

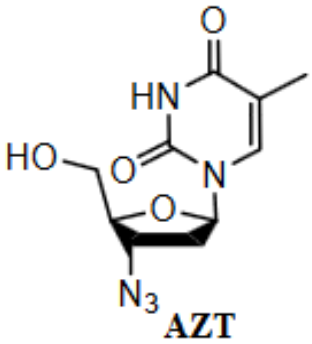

Figure 2. 2. Chemical structure of Azidothymidine. Adapted from (Menéndez-Arias and Gago, 2013)

Table 2.1: List of drugs approved by FDA (Food and Drug Administration) showing various targets and examples for treatment of HIV-1 infections. (adapted from http://www.fda.gov/).

NRTI	NNRTI	PI	EI
zidovudine (AZT)	nevirapine (NVP)	saquinavir (SQV)	enfuvirtide (T20)
didanosine (ddI)	delavirdine (DLV)	ritonavir (RTV)	maraviroc (MVC)
zalcitabine (ddC)	efavirenz (EFV)	indinavir (IDV)	
stavudine (d4T)	etravirine (ETV)	nelfinavir (NFV)	
lamivudine (3TC)		amprenavir (APV)	
abacavir (ABC)		lopinavir (LPV)	**INI**
tenofovir (TDF)		atazanavir (ATV)	raltegravir (RAL)
emtricitabine (FTC)		fosamprenavir (FPV)	
		tipranavir (TPV)	
		darunavir (DRV)	

2.2.2. Non-Nucleoside reverse transcriptase inhibitors (NNRTIs)

On opposite to nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs do not require conversion to their active form by the host kinases, and are considered noncompetitive inhibitors because they do not bind to nascent viral DNA, rather bind to the subunit of HIV and cause distortion and affection the chemical step of polymerization (Ravichandran et al.,2008). In this class, 5 drugs have been licensed by FDA, these agents include delavirdine, efavirenz, etravirine, nevirapine and rilpivirine and they show high specificity for HIV-1 but not to inhibit HIV-2 or any other retrovirus which makes it unique antiretroviral agents class (Famigliniand Silvestri,2016). Moreover, NNTRIs-based regimes are known to increase life span of individuals living with HIV, have less contraindication and better distribution which are some of the qualities made it to be Goldie as described by Esposito et al., (2012). The Major setback of NNRTIs are the rapid occurrence of resistance and cross-resistance because of type of mutation which can single or double amino acid mutations inside the NNBS of HIV-1 RT which affect the binding of the NNRTI (Delaugerre et al., 2001; Engelman and Cherepanov, 2012).

2.2.3. Protease inhibitors

Cheng et al., (2004) described this important class of antiretroviral drugs that attack HIV-1 protease (PR), an important enzyme needed in the proper assembly and maturation of infectious virions, thus made it a promising target for HIV infection medication. HIV protease, enzymes that is classified as aspartic proteases family and the arrangement of the molecule is a symmetrically assembled homodimer consisting of two identical subunits of 99 amino acids (Annemarie et al., 2010). Currently, there are about 10 drugs class which consists nelfinavir, saquinavir, atazanavir, ritonavir, tipranavir, indinavir, fosamprenavir, amprenavir, lopinavir, and darunavir (U.S.A., FDA, 2012). High specificity is required for these agents for them to be effective as expected. To now, all the HIV-1 protease inhibitors available, are known for targeting the active site substrate-binding groove of the homodimer enzyme, structurally a long cylindrical cavity that binds 6-7 amino acids through ionic, van der Waals, or H-bonding interactions (Donmienne et al.,2000). The enzymes-drugs complex allows the cleavage site at Gad and Gagpol proteins, however, there is little understanding of the exact mechanism of activation of viral protease, which is attached in the GagPol protein, although it is suggested that dimerization of *Gag-Pol* precursor proteins is essential (Annemarie et al., 2010).

Phillips (1996) succinctly explained the complex viral protease as polyprotein (Glycoprotein) precursor that is encoded by the *Gag-Pol* region of the viral genome. H1V proteases divide GP160 into GP41 and GP120. Cleavage of GP 160 into its component parts, protease promotes binding of GP120 of the virus to the CD4+ receptor site and fusion (gp41) of the viral coat with the CD4+ cell membrane.

This class of Anti-HIV made possible to use combination treatment with more than one class of drug which is popularly known as “highly active antiretroviral therapy” (HAART). Despite clinical benefit exhibited the drugs showed some poor pharmacodynamics by affecting distribution and function of hepatic cytochrome P450 3A system, also poor oral bioavailability, thus account for poor bioavailability.

Protease inhibitors are classified into two generations; Saquinavir, ritonavir, indinavir, and nelfinavir made up the first generation while amprenavir, lopinavir,

atazanavir, tipranavirs, and darunavir made up the second generation PIs.(Annemarie et al., 2010).

In the review, Annemarie et al., (2010) concluded that the mechanisms action of the first generation member of PIs was limited by poor bioavailability and high pill quantity, which affect the stick to dosage prescription and reduce long-term viral inhibition which prompted subsequent advance generation of protease inhibitors, second generation to redeemed effect of ritonavir metabolism and serve as a booster in the treatment of HIV.

2.2.4. Entry inhibitors

This class of drugs is considered as the newest arsenal in treatment of HIV infection, this class of antiretroviral has only one member called, enfuvirtide, it is known as earliest the fusion inhibitor, however, vast compounds are in the streamline of clinical development trial and indicating a promising treatment agent in few months or years (Briz et al., 2006). This step in viral replication cycle of HIV-1 into target cells involved multistep process of attachment, co-receptor binding, and fusion (Kuritzkes 2009; Henrinch and Kuritkes 2013). The agents have promising future as many are still under trial and shown significant activity. The predominant role played by the CCR5 co-receptor in the transmission and spreading of HIV makes this molecule the target of choice for blocking this mechanism. Kundru et al., (2008) explicitly described the role of CCR5 at attachment site for HIV, thus provide target for antagonist. The authors demonstrated that fraction of CCR5 molecules can be used as an antagonist and exhibited excellent antiviral effect both invitro and in vivo under clinical trials. A quaternary ammonium anilide (TAK-779) was the first small molecule CCR5 antagonist reported, but this compound was limited due to poor oral availability (Baba et al., 1999). Additionally, structurally similar compounds such as TAK-220 and TAK-652 are both in clinical trials showed good potency and/or pharmacological properties have also been reported by other pharmaceutical companies (Kundru et al., 2008).

2.3. Highly Active Antiretroviral Therapy (HAART)

The threat of HIV/AIDS to the global health sector has prompted stakeholders to look for ways to treat or stop progression of this disease, and prevent emergence of resistance. This concept involves use of combination of two or more classes of antiretroviral drugs to improve life-expectancy for those infected with HIV. Feeney and Mallon (2011) described the Conventional HAART involves use of three agents from three major drug groups; NRTIs and their analogues which inhibit the viral reverse transcriptase (RT) enzyme, NNRTIs - which also target the RT enzyme and PIs, which works against HIV protease. On which classes to be combined, factors such as CD4+ lymphocyte count, HIV load, potential toxicities, pill burden, drug interactions, age and HIV resistance have to be considered (Hammer et al., 2008). The use of HAART to reduce viral load in infected individuals, substantially led to increase in immunologic status and decreases complication of the syndrome. This "ray of hope" to HIV infected because it is known to elevate CD4 + cell levels, reduce levels of the viral RNA and increase AIDS-free life, for particular period. Moreover, the effect of ART in suppressing the virus may affect some major innate immune systems such inflammation and immune activation in such situation is considered as the cause of high rates of cardiovascular and other co-morbidities reported in HIV-infected individuals (Mataftsi et al., 2010).

The drugs in HAART regimen are categorized 'back-bone' and 'booster' for (NRTI), (NNRTI) and (PI) respectively. Sadly, complete eradication of HIV infection is far away from achieving with the present antiretroviral plans. At the early stage of HIV infection, the infected CD4 T-cells allow occurrence of latently, hence the persistence for long period, even with prolonged suppression of plasma viremia (Mataftsi et al., 2010). Despite significant effect in reducing viral loads by HAART, it is associated with some problems with the regimen, such as high toxicity, occurrence of resistance and high cost especially in developing countries or where no government effort to subsidize the drugs. Fellay et al, (2001) reported that significant number of individuals on antiretroviral therapy have some adverse reaction of the treatment.

2.4. Antiviral Agents Resistance

The major threat to HIV treatment is rapid and increasing emergence of resistance to available agents, which thwarted the effort to eradicate this pandemic. It has been stressed by many studies that high viral replicative loads, prolong exposure to antiretroviral drugs may increase selective pressure against viruses and natural mutation rates predispose to the emergence of antiviral resistance, thus the virus appear as persists or increasing viremia (Kimberlin et al., 1996; Imamichi, 2004; Strasfeld and Chou, 2010). Similarly, in a study by Laurent (2006), considered other factors such as lack of clinical and biological follow-up and drug supply irregularity and many patients interrupt their treatment especially in developing countries as driving force for increase emergence of resistance. The first drug used to treat HIV-infection, the Zidovudine, a member of NRTI developed resistance not long when it was licensed in early 1980 (Covens, 2010). Resistance to anti-HIV is reported in most of the drugs due to different types of mutation. (Covens et al. 2009). This indicates that no drug is spare by this challenge of resistance by HIV. To overcome the future challenge for treatment strategies, a rapid change in treatment plan is needed to halt further replication which allows natural selection of mutations, making the treatment process less effective. Furthermore, it is believed that it allows border cross resistance which limits future treatment plan.

Emergence of HIV drugs resistance has been extensively studied and special focus has been on HIV-type 1 variants (Sayan et.al.,2013; Chan, et al., 2015). The genetic nature of the HIV made it prone to rapid emergence of resistance, genetic factors such as rapid mutation, high turnover of viral populations and error prone possibilities have been reported by Sayan et.al., (2013). The authors analysed about 117 newly infected HIV patients and categorized them according standard set by European AIDS Clinical Society (EACS) Guidelines, using molecular technique (real-time PCR) the genes were sequenced and generated the phylogenetic tree. Furthermore, the authors analysed specific antiretroviral drug resistant mutation. In similar study by Sayan et. al., (2012) antiretroviral drug resistance in naïve patients in Turkey was determined and found total of 11.8% of all the patients studied (59) to have significance HIV-1 primary drug resistance. In a study conducted in Brazil among 400 patients for the

antiretroviral drug resistance to HIV and the results showed 7% of patients that have not started receiving ART treatment are confirmed to have carried the viruses with one or more major mutation associated with drug resistance and on individuals class of antiretroviral agents, NNRT has the highest resistance (4.4%), followed by NTRIs (1.3%) and PIs (1.0%) (Sprinz et al., 2009).

This agreed with previous study by Rolando et al., (2006), that showed a significant mutation causing HIV drug resistance were present, the main class of drug was NNRTI with K103N mutation whereas the rest of anti-HIV classes were minimum. In both studies a major risk of poor response was revealed regardless of the sample size, thus there is need to apply the resistance test to all new adult patients diagnosed with the virus before commencement of antiretroviral therapy in present guidelines. There exist many reports on the prevalence of HIV-1 drug resistance.

There is a variation in the different study area which is attributed to study population, geographical area, other factors such detection and interpretation of results. Globally, the major area of high reports of resistance are reported in region with ART usage records such Western Europe, North America and some part of South America. Booth et. al., (2007) attributed the drivers of the resistance in such places due to not taking full dosage, adherence to prescription and less tolerance that result to drug resistance in such newly patients and further spread of the resistant type of the virus.

There exist three main mechanisms of resistance to drugs by HIV as reported by (Taiwo, 2009).

(1) De novo resistance mechanisms: The classical resistance mechanism exhibited main by HIV type 2 to NNRTIs. This is favoured by the nature of polymorphism of subtype B and non-subtype B HIV-1 by a particular mutation. There is variation of polymorphism in the transmitted variants drug resistance, although it may serve other function such as accessory role or giving simple way of the actual type of resistance.

(2) Darwinism mechanism: Resistant variants are formed due high replication nature of HIV lifecycle which leads to rapid point mutation. Usually the translational error

occurs at a specific codon, hence a defective virion is formed which is the responsible for the resistance (Fig. 2.2)

(3) Transmitted drug resistance: ARV primary resistance by HIV can be transmitted resistance or through infection with a drug-resistant HIV strains. The transmission of the resistance happens via sexual, parenteral and vertical routes of viral acquisition.

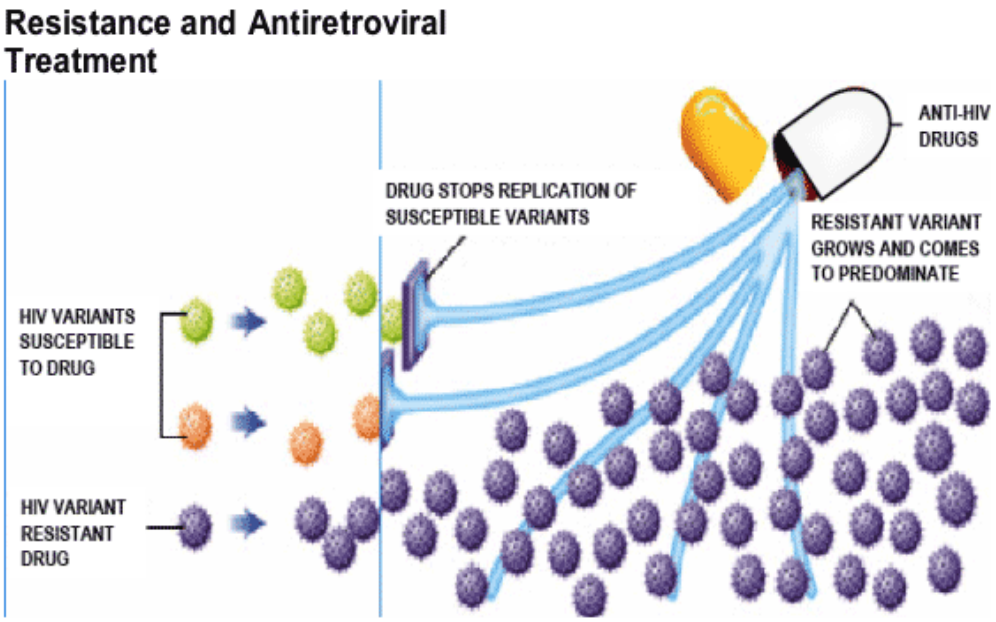

Fig. 2.3. Showing natural selection of antiviral resistance. Adapted from (Menéndez-Arias and Gago, 2013).

2.5. Occurrence Of Natural Resistance to HIV

HIV infection does not automatically lead to the development of the syndrome; AIDS in some set of individuals, strikingly, there are some exception, a set of people do not progress from HIV infection to AIDS to complete development of AIDS, despite being infected with the virus. These group of individuals are called slow or long-term nonprogress (LTNPs) and they are considered have natural immunity against the virus and such individuals have ability to maintain the HIV viral load titer to a lowest level (Kumar 2013). The rate of disease progression rates differs, but

majority of people progress to the advanced stage of the infection. The timeline of the progression is usually categorised into three: *(i)* rapid progression, here, the AIDS develops within 3 years after the initial infection; *(ii)* intermediate progression, in this group, the AIDS develops slowly in range of 3 to 10 years after seroconversion; and *(iii)* long-term non-progression (LTNP) in this category, the infected individuals maintain high CD4+ and CD8+ lymphocyte counts and remain therapy naïve (Zeller et. al., 1996; Grabar et.al., 2009; Kumar 2013). It can be deduced that the presence of high level of immune cells in such individuals largely contributed to such genetic tolerance, hence halt the HIV progression to AIDS.

The period to determine the non-progression of HIV positive varies, as suggested by different studies, with different duration, thus makes classification into this group difficult. Different range of period have been used for the classification, some adopt >8 years duration to classify the non-progression while others adopt>10 years (Ashton et. al.,1998; Grabar et. al. 2009). However, it is clear that this group of HIV positive patients is less reported by Kumar (2013) to be around 5% of total of individuals with HIV. Furthermore, the availability of viral load testing help subdividing such set of people into 2: (1) Presentation of low detectable virus in blood at concentration of <5000 HIV-RNA copies/ml, termed long-term non-progression; (2) Presentation of HIV-RNA values showing plasma HIV-RNA below 50 copies/ml, and called “elite” or “natural controllers” (Kumar 2013). This phenomenon is influence by a genetic advantage of people carrying particular gene called CCR5-Δ32 gene (Sabeti et al., 2005).

The CCR5-Δ32 allele is functioning to give protection against HIV and also smallpox, such allele is present in 10% of the Northern Europe population (Sabeti et al.,2005). In another study in which some people show ability to control HIV infection is reported by Migueles et al., (2000), where presence of particular Human leukocyte antigen (HLA) gene types such as HLA-B*5705 and HLA-B*2705 confer ability to control HIV infection to certain degree. Rhodes et al., (2000) reported another group of people that are carrying a gene, Nef-deleted variants, which interfere with HIV replication in a study conducted among Australians cohorts group. There are also natural limiting factors to the viral replication process such as APOBEC3F/G active

against Vif and Teftherin active against Vpu can also stop HIV-1 viral replication. Members of Tripartite Motif (TRIM) protein family such as TRIM5α and TRIM22 are considered to have restriction effect in retroviruses (Barr et al., 2008).

The TRIM family is component of innate immune system of the host. constituent part. However, TRIM5α is not effective specifically against HIV-1, but it can work on murine leukaemia virus reduction, on contrary TRIM5α has effectively stop HIV-1 infection in rhesus macaques and old world monkey. Another natural host resistance by TRIM22 is reported to have down-regulates transcription from the LTR and prevent important viral replication step, viral assembly by stopping HIV-1 gag transport from the nucleus (Stremlau et al., 2004; Yap et al., 2004; Barr et al., 2008; Kumar 2013).

There are attempting to study the gap between long-term non-progressive infection to progressive HIV-1 disease base on identifying host, genetic and viral factors which play role as an indicator of disease progression (Kumar, 2013).

2.6. Host And Genetic Factor

The progression of HIV infection to AIDS can be understand based on the genetic factor encoded and are classified into three: *(i)* genes within human leukocyte antigens (HLA) that regulate host immune response to infection; *(ii)* genes encoding cell-surface receptors or ligands for these proteins; *(iii)* other cytokine or immune response genes. In this category, the diversity encoded by individuals determines the period of progression, for instance the difference at HLA class I loci (HLA-A, HLA-B and HLA-C) is strongly correlated with resistance and conversion to AIDS (Ntale, et.al., 2012).

2.7. Viral Factor

Another important concept that determine progression of HIV is the amino acid sequence of the virus, for example, Carl et.l al., (2000) reported that Amino acids sequence play role in an invitro studies and found that Nef is correlated with the viral pathogenicity of HIV-1 in vivo. The authors showed that Nef proteins derived from LTNPs and slow progressions (SPs) were lacking ability of promoting viral replication, infectivity or even both. The point of deletions in the nef/LTR region

continually increased progressively follow up period. It can be deduced that there are many factors that involve in the disease conversion from infection to syndrome.

2.8. Detection Of Resistance

One of the easy ways to monitor efficacy of agents is by monitoring the susceptibility and resistivity of the virus to the available agents. Two distinct methods are employed for resistance testing; genotypic or phenotypic technique (Covens et al. 2009)

a) Genotypic assays

This technique has advantage of being rapid and less expensive for determination of resistance which involved detection of mutations in the constituent of the viral genetic make up by direct sequencing of the point of interest for the antiviral agents after amplification of the gene using real time PCR. The viral RNA is sourced from patient plasma. The major setback for this method is the issue of result interpretation.

The effectiveness of the other drugs targeting same point is not affected but may result to high susceptibility, cross resistance or ressentized certain type of drugs. (Covens et al. 2009). Although this method is complex, but it is preferred due to its ability to detect resistance genes in a mixed sample and low mutation level, hence it is more sensitive and gives clue for potential resistance to emerged (Shafer 2002).

The genotypic technique is considered as a qualitative method which involves indirect measuring of viral susceptibility to antiretroviral agents based on sequence of the viral genome to detect mutation. The viral genome sourced from the patients is then compared to the database HIV sequences to generate the resistance pattern, these can be found at (http://hivdb.stanford.edu) (Jacobes et al., 2008).

b) Phenotypic assays

This assay involves the use of various concentration of anti-HIV agents to detect the inhibition of replication process of the virus. This method measure the specific concentration of the drug to inhibit virus life cycle by 50% (EC50). The interpretation of result of phenotypic testing determined as a fold change in EC50 for a given

inhibitor of the tested virus in comparison to a fully susceptible laboratory control isolate. A fold change in EC50 grater than 1 theoretically indicates reduced susceptibility towards the tested inhibitor with different interpretation cut-offs(Covens et al.,2009).

This method considered as direct and conventional technique because it measures the virus ability to replicate in the presence of an agents(antivirogram).

The measurement is qualitative where degree of resistance is quantified. In this testing, as indicated by phenosense GT system, the mechanism is basically assessing the activity of the virus in the presence and absence of a drug to be tested. This technique compares the concentration, usually 50% inhibitory (IC50) of drug required to inhibit the clinical isolates with that of wild type reference strains. It is considered laborious and time consuming, but the activity of the viral enzymes can be measured which will reflect the susceptibility of the virus to the antiretroviral agents (Jacobs et al.,2008).

In both methods, the logic behind is to indicate treatment failure, although genotyping is most favourable test for the patients undergoing routine follow-up because of lower cost and faster in time (Vercauteren and Vandamme,2006).

European HIV Guidelines Panel (www.europeanaidsclinicalsociety.org) suggested the susceptibility testing to both naive and experienced patients as recommended by international AIDS society-USA panel (Kagan et al,2004). However, there is complexity in genetic interpretation especially in genotypic method, and the chief reason is the genetic make up of the mutation which can differe between patients and the subtypes and hence can impact the phenotypic presentations of the previously revealed mutations, and also result in variation in resistance method and selection of new mutations by selective pressure (Abecasis et al., 2005).

3.1. MATERIALS AND METHODS

The current study was established at the laboratory of Kocaeli University Hospital (Turkey) during the period between January and October 2018. An approximately 103 samples were obtained from different cases were newly diagnosed with HIV-1 all over Turkey. For each patient an ethical consent was gained from the research ethic committee at Kocaeli University and written in an informed agreement.

According to the Guidelines of European AIDS Clinical Society (EACS), every patient was classified as an HIV carrier. All data recorded at the Turkey Ministry of Health showed that each case in the present study was newly diagnosed and was an ART-naïve. Based on the guidelines provided by the Centre for Disease control (CDC), different stages of HIV infection were evaluated.

Blood samples were collected with carful from all patients Using (K_2EDTA) tubes, subsequently samples were harvested, and the obtained plasma was stored at -80°C until use. By using microparticle enzyme immunoassay commercial kits (Axsym; Abbott Laboratories, Abbott Park, IL, and Elecsys, Roche Diagnostics, Mannheim, Germany) Anti-HIV-1/2 antibody was examined. In Istanbul Venereal Diseases Hospital/ Turkey both ELISA and Western blot test (DIA PRO, HIV-1 LIA, Diagnostic BioprobesSrl, Milano, Italy) were used and confirmed that all anti-HIV were positive. As well as a unique coding number was given for each sample to maintain subject confidentiality.

3.2. HIV-1 RNA quantification

Using commercial PCR assay (Qiagen GmBH, Hilden, Germany) was used in this study for viral RNA detection and quantification, and Abbott M2000 SP/ Abbott Real Time HIV-1 Amplification Kit (Abbott Molecular Inc., Des Plaines, IL).

3.3. Sequencing of HIV–1 *Pol*

The viral resistance was evaluated through performing a genotypic resistance method by sequence analysis of the viral protease and portion of the reverse transcriptase (RT) using a house keeping gene. Finally, guidelines provided by ANRS (AIDS National Research Agency) were used for designing specific primer pairs and adopted by Sayan et al., (2016) to read the results.

The polymerase chain reaction (PCR) conditions has the following details :RT (codons 41– 238): outer primers (798bp); MJ3:5′-agtaggacctacacctgtca-3′ (2,480 to 2,499) and MJ4:5′-ctgttagtgctttggttcctct-3′ (3,399 to 3,420), inner primers (573bp) A(35): 5′-ttggttgcactttaaatttt cccattagtcctatt-3′ (2,530 to 2,558) and NE1(35): 5′-cctactaactt ctgtatgtcattgacagtccagct-3′ (3,300 to 3,334). Sequencing primer; A(20): 5′-attttcccattagtcctatt-3′. Protease (codons 23–90): outer primers: 5′prot 1:5′-taatttttagggaagatctggccttcc-3′(2082-2019) and3′prot 1:5′-gcaaatactggagtattgtatggatttt cagg-3′ (2,703 to 2,734), inner (amplification: 507-bp fragment) and sequencing primers 5′ prot 2: 5′-tcagagcagaccaga gccaacagcccca-3′ (2,136 to 2,163) and 3′ prot 2:5′-aatgctt ttatttttttcttctgtcaatggc-3′ (2,621 to 2,650). HIV-1 cDNA synthesis was done with the First Strand cDNA Synthesis Kit (Thermo Scientific Inc., Fermentas, Lithuania) including the M-MuLV reverse transcriptase enzyme. The PCR cycle used was 95℃ for 10 minutes, and then 45 cycles consisting of 95℃, 55 ℃, 72℃ with time of 45s, 45s and 45s respectively. Purification of all PCR products was conducted using the Highly Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany) and directly sequenced with ABI PRISM 310 Genetic Analyzer equipment using the Dynamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ). The following thermal protocol was employed for the cycle sequencing: 35 cycles of 95℃for 20 s, 50℃ for 25 s, and 60℃ for 2 min.

Electropherogram was used to obtained the sequences using Vector NTI v5.1 software (InforMax, Invitrogen, Life Science Software, Frederick, Md., USA).

3.4. Antiretroviral Drug-Resistant Mutations Analysis

HIV-1 antiretroviral drug-resistant mutations were analyzed according to WHO recommendation guidelines for the tracking of drug resistant mutations. The WHO SDRM list included only consensus nonpolymorphic drug resistance mutations at 43 genomes in HIV-1 protease and RT. Selected mutations were defined as those occurring at a prevalence 0.5% in ART naïve individuals in subtypes for which >1,000 sequences were available. Then the algorithm of HIVdb-Stanford University (www.hivdb.stanford.edu/) was used to interpret antiretroviral drug resistance mutations. The data generated was then compared to the consensus subtype B reference sequence, and the differences were used suspect the parameters to interrogate the HIV drug resistance database as previously reported by Sayan et al., (2013).

3.5. Statistical analysis

SPSS was used to analyse the data. Windows statistical software v20 (SPSS Inc., Chicago, III., USA). The descriptive analyses for the characteristics as well as clinical information of the patients were analysed, and independent proportion test was conducted using Chi-square Statistic with significant level of $P \leq 0.05$.

4.1. RESULTS

An analysis for drug resistant mutations was carried on 103 blood samples. These samples were acquired from ART-naive patients who were infected with HIV1. The results of demographic details of the sample population is presented in table 4.1. Majority of the patients were Turkish nationals (93, 90.2%) while non-Turkish (10, 9.7%) of the age the mean is 32.5 years with the proportion of gender 82.5 % male and 17.4 % female represent 85 and 18 respectively. Other characteristic details include CD 4+ T cell counts, and the results show a mean of 367.0mm^3, subsequently the CD4+ loads were further classified as early and late presenter (Table 1). In the present study, the early presenter (>350) mean is 523.5mm^3 while the late presenter (≤350) is 252 mm^3. The following laboratory and clinical criteria were also considered; mean of HIV-RNA load is 1.79+E5 IU/ml, pathway of acquisition is high in heterosexual (62, 60.19%), then in order of decreasing manner MSM (29, 28.1%), bisexual (9, 8.7%), intravenous drug users (2, 1.9%), and blood transfusion (1, 0.97%). The prevalence of HIV and other co-infections was evaluated, and the results were also presented in table 4.1. At this stage, large percentage of the patients had no co-infection (91, 88.34%) however, the prevalence of HVB 4.8%, while syphilis 3.8%, oral candidiasis, HVC, *H. pylori*, and Toxoencephalitis were all 0.97%.

The frequency resistance of individuals' class of antiretroviral agents was analysed and the results are shown in Table 2. The incidences of primary drug resistance for NRTIs, NNRTIs and PIs were 7.76% (8/103), 22.3% (23/103) and 0.97% (1/103) respectively. The difference between drug groups (NRTI, NNRTI and PI) resistance incidents were significant at (χ^2= 25.889, p<0.001). It can be inferred that more resistance could be attributed to NNRTI drug users while more sensitivity can be attributable to PI drug.

Table 4.1. Demographic and clinical characteristic of naive ART HIV-1 infected patients (n=103)

Variables	Median (min – max)	n (%)
Age (yrs)	32.50(17.00–74.00)	-
Nationality		
Turkish		93(90.29%)
Non-Turkish		10(9.71%)
Sampling, region/city of Turkey	Marmara/Istanbul,Kocaeli,sakarya,Bursa Black sea/Samsun,Trabzon,Bolu,Ordu Southeast Anatolia/Gazianatep,Elazig Central Anatolia/Sivas Aegean/Izmir, Denezili, Canakkale Mediterranean/Isparta,Mersin	
CD 4+ T-cell count (mm^3)	372.50(3.00-1144.00)	
Early Presenter (>350)	252.00(3.00-348.00)	47(45.63%)
Late Presenter (≤350)	520.00(354.00-1144.00)	56(54.37%)
HIV-RNA load (IU/ml)	1.78+E5 (520.00-4.5+E8)	
Acquisition Path		
Heterosexual		62(60.19%)
MSM		29(28.16%)
Bisexual		9(8.73%)
Drug Users		2(1.94%)
Blood Transfusion		1(0.97%)
Co-Infection		
HBV^+		5(4.85%)
Syphilis		4(3.88%)
HCV^+		1(0.97%)
Toxoensefalitis		1(0.97%)
Oral Candidiasis		1(0.97%)
PCP		1(0.97%)
H.Pylori		1(0.97%)
Unknown		92(88.46%)

Turkish patients with antiretroviral naive were evaluated for the presence of primary antiretroviral resistance mutations in HIV-1, and the result is presented above in table 4.2. The specific mutation pattern of the antiretroviral agents was assessed according to guidelines set by WHO 2009 SDRM and the following groups of the drugs tested; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 4.2. ART resistance pattern

Characteristic/drugs		Percentage
NRTI		
	Sensitive	96(93.20%)
	Resistance	7(6.80%)
NNRTI		
	Sensitive	81(78.64%)
	Resistance	22(21.35%)
PI		
	Sensitive	102(99.02%)
	Resistance	1(0.97%)
Mutation patterns		
NRTI	A62V,F77L,K70KR,M41L,T215S	8(7.76%)
NNRTI	E138A,E138G,K103N,V179D,K103KN,K103T	23(22.3%)
PI	L24LI	1(0.97%)

HIV-1 subtyping results are shown in three categories (Subtype B, non-subtype B, and CRF). In accordance with the results, subtype B was dominant 58 (56.31%), non-subtype B had 34 (33.0%) and CRF had 11 (10.67%). As presented in table 4.3.

Table 4.3. HIV-1 SUBTYPES

HIV-1 SUBTYPE		N (%)
Sub-Type B		58(56.31%)
Non-Sub -Type B		34(33. 0%)
	A1	20(19.41%)
	F1	11(10.67%)
	G	2(1.94%)
	C	1(0.97%)
Circulating recombinant factor (CRF)		11(10.67)
	B+CRF02_AG	8(7.76%)
	CRF02_AG	2(1.94%)
	CRF07_BC	1(0.97%)

5.1. DISCUSSION

HIV is still major killer across many countries in the world. According to WHO statistics, it has result to dead of more than 35 million people. In 2017 alone, the number of people who died from HIV related causes were 940,000 worldwide (WHO, 2017). In addition, 36.9 million individuals were HIV positive at the end of 2017, with another 1.8 million newly infected. It has been projected that around 52% of children and 59% of adults were under antiretroviral therapy (ART) in 2017, the amount of HIV positive patients in Turkey has increased dramatically by about 465 percent in the last decades, reaching 14,695, according to Health Ministry report. This goes contrary to the global report of HIV declining; this is alarming and great threat to the health economic implication of country (www.hatam.hacettepe.edu.tr). Majority of the cases are detected in foreigners (16.5%), in addition the statement issued on world AIDS day 2016 provided detailed data at 1,661 HIV and 73 AIDS cases.

Antiretroviral therapy (ART) is the application of HIV drugs in order to treat infections caused by HIV. ART is recommended to those tested HIV positive. ART can't cure HIV completely, but the agents enhance the life of people infected by HIV helping them to live lengthier and better lives. The transmission of HIV is reduced by these agents. This help in prolonging life of HIV positive patients. However, ART is being challenged by rapid development of resistance. The present therapy procedures recommend that treatment regimen be introduced at a CD4+ lymphocyte count of 350 cells per mm^3 or under. Pregnant women should undergo a prevention program, while infants under the age of one should be given treatment, irrespective of their CD4+ count.

In the West, a resistance status is encouraged on all HIV-1 positive patients before the introduction of ART as around 10% of patients carry primary drug resistant strains (Salzberger et al., 2005). In this study, the patients that have not experienced ART exposure in HIV-1 in adult patients in Turkey from January to October 2018. The results indicate that vast numbers of HIV-1 patients in the study were Turkish indigenes (90.4%), contrary to the previous report (Ersan 2017), this can be explained by the sample sizes of 103 that involved in our study. For the commencement of

treatment, the patients were tested for the CD4+ counts and the results revealed that early presenter category had 54.8% while late presenters 45.2%; such investigations into the decline of CD4+ count in HIV patients not undergoing ART, is essential in the proper clinical management of HIV. Interestingly, previous studies indicated decline in CD4+ count in patients before start ART regimes (Patrikar et al., 2013). Patrikar et al., (2013) reported decline in CD4+ count in a study conducted amongst 209 patients in India who had not received ART showed decline CD4+ count over a certain period of time; a decrease from 1000 mm^3 to780 mm^3 was observed within six months and further decline was reported, this comes in agreement with the current study. Similarly, in the TREAT Asia HIV observational database (TAHOD) a study was carried out in 1676 among Asian patients and indicated decline in CD4+ during months 6–12 of 21.5, while in months 18–24 it was 25.8 and in month 24 and above it was 59.1 cells (Jialun et al., 2010). It is clear that the longer the time to measure the CD4+ the higher decline rates. Measurement of degree of immunosuppression is important because it will a determinate point for commencement of treatment and degree of disease progression. In another study conducted in Nigeria amongst naive patients showed variation of CD4+ counts amongst the subjects (Akinbami et al., 2012). The authors reported the mean CD4+ counts in HAART-naïve, HIV positives results were higher in male than female and an overall mean of 298.76 ± 246.93 cells/mm^3. This could be compared between males and females and an overall mean CD4 count of 306.65 ± 232.24 cells/mm^3. Despite the variation among the gender and ages, overall, there were decline in the counts.

In another concept, this study looked at the routes of transmission amongst the subjects, and the results indicated that the heterosexual presented higher (60.6%) as mode of acquisition than the MSM (27.9%), bisexual (8.7%), and intravenous drug users (1.9%) with least by blood transfusion (1%). Strikingly, our study agrees with report of (Agacfidan et al., 2014) in that the two main means of the transmission of HIV/AIDS in Turkey are heterosexual contact (48.9%) and homosexual contact (8.9%). This differs substantially with Europe where HIV/AIDS is mostly transmitted through risk behaviour among homosexuals. This can be explained by the differences in socio-cultural states of the populations, in Turkey, mostly the sex workers are considered as the driving factor. Several studies have reported route of HIV infection

in the Middle East, which is combined with North-Africa due to similarities in socio-cultural relations and tagged as Middle East and North Africa (MENA) region (Abu-Raddad et al.,2010; Mumtaz et al.,2014).This region comprises of a geographically defined combination of countries which include high-income well-developed nations and low to middle income countries and may present different routes of transmissions. The studies disclosed that injecting drug users, men who have sex with men (MSM) and to smaller degree female sex workers, this is in contrary to our finding that intravenous drugs users are higher in the study area despite being close to MENA region. However, in an extensive review on HIV trends in MENA countries by Gökengin et al., (2016) confirmed that in the MENA region sexual transmission appeared to be number one route for infection, other than other means, this goes in agreement with the current study.

The main feature of HIV infection is its ability to lower immunity which could serves as a gateway of other infections, thus co-infections are characteristic that HIV infection is progression to full blown AIDS syndrome. In this study, HIV co-infections to the samples were also investigated. The results revealed small percentages of the patients have co-infection (Table 1). This can be explained by the fact that the patients are at their early presentation of the HIV stages. However, the result showed PCP, oral candidiasis, *Hpylori* and HCV about 1% each. Infection such as oral candidiasis is an indication that AIDS has occurred in HIV infected person, hence a last stage of opportunistic diseases. According to WHO, HBV infection is more associated with HIV as a co-infection because of these shared modes of transmission. Also, Hepatitis C virus (HCV) shares the same route of transmission via blood virus (i.e., direct contact with the blood of an infected person). Furthermore, HCV is estimated to affect 2–15% of people with HIV around the world (90% of which are people who inject drugs (PWID)) and a further 5–20% are estimated to have chronic HBV infections (WHO, 2017).

5.2. HIV-1 Subtypes

HIV use mutational methods to evade immune system, hence results to the diversity of the subtypes which reflects the genetic evolution and allow the emergence of new HIV strains. Generally, group M is considered as the predominant circulating

HIV-1 group. It further divided into subtypes, denoted with letters, and sub-subtypes, denoted with numerals. Subtypes A-D, F-J and K are currently recognized. HIV-1 subtypes, also called clades, are phylogenetically linked strains of HIV-1 that are approximately the same genetic distance from one another; in some cases, subtypes are also linked geographically or epidemiologically. The variation of the genetic within a subtype can be 15 to 20%, whereas variation between subtypes is usually 25 to 35% (Barbara et al., 2008).

This research, the molecular investigation of HIV-1 subtypes revealed that subtype B (55.7%) and non-subtype B specifically A1 (19.2%) as the predominant subtype in the study area. Another subtype was non-subtype B F1 (10.6%), G (1.9%) and C (1%) (Table 4.3). Historically, the first work to determine HIV-1 subtypes was conducted by Yilmaz et al., (2006). The authors sequenced *env* of 27 HIV/AIDS patients with no clear treatment status and the result revealed that HIV-1 subtype B as the most dominant in Turkey, this is in agreement with this research. In a study by Sayan et al., (2013) indicated the high dominance of subtype B (52.1%) and interestingly as a case of follow up of the genetic diversity amongst the Turkish immigrant in Germany, a study by Schulter (2011) found same subtype B as the dominant subtype. The presences of subtype B has been reported in many countries such as, Brazil (Leal et al., 2010), Australia, Europe, and the Americas (Hemelaar et al., 2011).

However, HIV-1 subtype C is presently the most common HIV-1 subtype globally, this has resulted in international efforts in the development of a subtype C candidate vaccine (Jacobs et al., 2008). It was in the 1980s that HIV-1 subtype C was first discovered in North East Africa, namely, Malawi and Ethiopia (Jacobs et al., 2011). It can be deduced that the HIV-1 subtype diversity is region specific; however, the global movement and rapid emergence of mutation and spread of HIV help in occurrence of unusual subtypes in a particular region. The worldwide circulation of different subtypes and the spread of recombinant forms reflex the complexity of the molecular epidemiology of HIV-1, and in turn make the development of one type of vaccine a complex work. For the unique characteristic of HIV-1 subtype C, Alien et al., (2005) reported HIV-1 subtype C differentiate from the HIV-1 subtype for example

in the presence of extra NF-κβ enhancer copies in the LTR, Tat, Rev prematurely truncated protein and 15 bp at the 5 ends of Vpu reading frame. Subtype C also has a relatively conserved *env*gp120 V3 loop, with the virus showing preference to using CCR5 as its major co-receptor irrespective of the stage of disease progression.

Another subtype investigated in this study is CRF, it was found that B+CRF02_AG (7.69%), other circulating strains of CRF are CRF02_AG (2.9%) and CRF07_BC (1%). Identification of CRFs in Turkey was first reported by Sayan et al., (2013) and the results revealed that CRF01_AE (5.1%) is the predominant, similarly Hemelaar (2011) found CRF 02_AG was prevalent in West and Central Africans, and Middle Eastern/North Africa, also CRF01_AF detected in South-East Asians, East Asians and Central Africans, other distributions of CRFs such as CRF03_AB found in Eastern Europeans and Central Asians while CRF12BF in South Americans.

5.3. HIV- 1 Drug Resistance Patterns

This study analysed HIV-1 drug resistance pattern in navies patients. ART dramatically help in keeping lower viral count. Therefore, life expectancy of patients treated by ART has substantially improved, with therapy plans continually improving. However, due to therapeutic drugs creating selective pressure, HIV develop mutations, which in hand create a resistance to ART drugs (Johnson et al., 2003; Thompson et al., 2010).

The prevalence of ART in our study revealed the following NRTIs, NNRTIs and PIs are presented as follows; NNRTIs 23 (22.3%), NRTIs 8 (7.76%) and PIs 1 (0.97%). The transmission of HIV-1 drug resistant variations can easily infect and spread within a given population, hence testing at molecular level for antiretroviral resistance is recommended (Bennett et al., 2009). HIV-1 antiretroviral drug resistance testing of patients not responding to NNRTI-therapy was reported (Ndembi et al., 2011). In a surveillance of HIV-1 drug resistance under European society called SPREAD, particular study investigated transmission of drug-resistant strains among 2793 naive patients across 20 EU countries and Israel, the results showed that the occurrence of transmitted drug resistance to be 8.4% and breakdown of individual class of drugs revealed that NRTI had 4.7%, NNRTI had 2.3% while PI 2.9% (Vercauteren et al.,

2009). This is in contrast with the present study with high prevalence of NNRTIs (21.2%), this difference could be explained by the small sample size of the current study while the study of Vercuteran et al., (2009) involved large population from different countries.

Specific mutation for each group of drug resistance was assessed in this study, and it revealed the following; In the NRTI, 3 A62V (2.91%), 2 M41L, 1 F77L (0.97%), 1 K70KR (0.97 %) and 1 T215S (0.97%) drug resistance mutations were found. In the NNRTI, 13 E138A (12.62%), 3 E138G (2.91%), 3 K103N (2.91%), 2 V179D (1.94%), 1 K103KN (0.97%) and 1 K103T (0.97%) while in PI drug, one L24LI (0.97%) drug resistance mutation was found in table 4.2. Previously, the prevalence of A62V among ART-naive patients in Turkey was tracked for the fact that the north-eastern neighbours of Turkey showed the high prevalence in Eastern Europe (Carr et al., 2005; Sayan et al., 2013). The presence of A62V mutation in this study is an indication of possible transfer case or an indication of dominance in the region. Interestingly, A62V is known to occur in NRTI-resistant, a study by Carr et al., (2005) reported such mutation and related it HIV-1 sub-type A in intravenous drug users. Strikingly, the high prevalence of E138A (12.62%) was not reported previously in the study area. This should be another tracking point for drug resistance mutation

5.4. CONCLUSION

Conclusively, this study confirmed the presence of antiretroviral drug resistance in naïve HIV-1 infected individuals in Turkey, clearly such a high rate of NNRTIs is alarming. Specific resistance mutation genes were detected which alarm natural resistance among the naive HIV-1 infected patients. However, the study is restricted by the small sample size and some of the data could not be established. The study recommends that larges screening of all suspected HIV-1 individuals to be subjected to molecular detection of drug resistance patterns before treatment commenced.

REFERENCES

Abbas A, Lichtman A, Pober J. Cellular and Molecular Immunology. W.B. Saunders: Philadelphia. 2000.

Abecasis AB, Deforche K, Snoeck J, Bacheler LT, McKenna P, Carvalho AP, Gomes P, Camacho RJ, Vandamme, AM. Protease mutation M89I/V is linked to therapy failure in patients infected with the HIV-1 non-B subtypes C, F or G, Aids. 2005; 19:1799-1806.

Abu-Raddad LJ, Hilmi N, Mumtaz G, Benkirane M, Akala FA, Riedner G, Tawil O, Wilson D. Epidemiology of HIV infection in the Middle East and North Africa AIDS. 2010;24 Suppl 2:5-23.

Agacfidan A, Kaiser R, Akgul B. HIV in Turkey, a country bridging the Islamic world and Europe. Journal of infection and public health. 2014;7:249-250.

AIDS Cases and its Carriers in Turkey Distribution by years. Available at http://www.hatam.hacettepe.edu.tr/veri-ler_Haziran_2013.pdf (last accessed January 2019)

Akimoto H, Kaneko H, Sekigawa I, Kaneko Y, Yamamto N. Binding of HIV-2 envelope glycoprotein to CD8 molecules and related chemokines production Immunology.1998;95:214-218.

Akinbami A, Dosunmu A, Adediran A, Ajibola S, Oshinaike O, Wright K, Arogundade O. CD4 Count Pattern and Demographic Distribution of Treatment-Naïve HIV Patients in Lagos, Nigeria. AIDS research and treatment. 2012; 2012:352753.

Analysis of the treatment cost of HIV/AIDS in Turkey. Available from: https://www.researchgate.net/publication/299547488_Analysis_of_the_treatment_cost_of_HIVAIDS_in_Turkey [accessed Dec 08 2018].

Annemarie MJ, Wensing, Noortje M. van Maarseveen, Monique Nijhuis. Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. Antiviral Research.2010; 85:59–74.

Ariën KK, Abraha A, Quiñones-Mateu ME, Kestens L, Vanham G, Arts EJ. The replicative fitness of primary human immunodeficiency virus type 1 (HIV-1) group M, HIV-1group O, and HIV-2 isolates. J Virol . 2005;79: 8979-8990.

Baba M, Nishimura O, Kanzaki N, et al. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci U S A. 1999;96:10:5698-5703.

Bean B. Antiviral Therapy: Current Concepts and Practices. Clinical microbiology reviews.1992; 5:2:146-182.

Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug resistance: 2009 update. PLoS ONE.2009;4:e4724, https://doi.org/10.1371/journal.pone.0004724.

Booth CL, Garcia-Diaz AM, Youle MS, Johnson MA, Phillips A, Geretti AM. Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection. J Antimicrob Chemother. 2007; 59: 517-524.

Briz V, Poveda E, Soriano V. HIV entry inhibitors: mechanisms of action and resistance pathways. Journal of Antimicrobial Chemo therapy.2006;57:4:619-627.

Cameroon. Emerging Infectious Diseases • www.cdc.gov/eid • 12006;2;61:01-103.

Carr JK, Nadai Y, Eyzaguire L et al. Outbreak of a West African recombinant of HIV-1 in Tashkent, Uzbekistan. J. Acquired immune Defic. Syndr. 2005;39:570-575.

Chan PA, Hogan JW, Huang A, DeLong A, Salemi M, Mayer KH, Kantor R. Phylogenetic Investigation of a State wide HIV-1 Epidemic Reveals Ongoing and Active Transmission Networks Among Men Who Have Sex With Men. Journal of acquired immune deficiency syndromes. 2015; 70:4: 428-435.

Cheng TJ, Brik A, Wong CH, Kan CC. Model system for high-throughput screening of novel human immunodeficiency virus protease inhibitors in *Escherichia coli.* Antimicrob Agents Chemother.2004;48:7:2437-2447.

Christian L, Charles KL, Michèle T, Léopold Z, Nathalie Noumsi, Christelle B, Anke B, Eitel M, Sinata KS, Martine P, Eric D, Antiretroviral Drug Resistance and Routine Therapy, Cameroon. Emerging infectious diseases. 2006;12:6:1001-1004.

David WK, Richard JW. Antiviral resistance: mechanisms, clinical significance, and future implications. Journal of Antimicrobial Chemotherapy.1996; 27:6:403-421.

Delaugerre C, Rohban R, Simon A, Mouroux M, Tricot, C, Agher, R, Huraux, J.M, Katlama, C, Calvez, V. Resistance profile and cross-resistance of HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor-containing regimen. J. Virol. Med. 2001; 65:445–448.

Deniz G, Fardad D, JohnnyT , Ben C, Navid M, Donmienne L, Giovanni A, David PF. Protease Inhibitors: Current Status and Future Prospects. 2000;43:3:305-341.

Engelman A, Cherepanov P. The structural biology of HIV-1: mechanistic and therapeutic insights. Nat. Rev. Microbial.2012; 10:4:3: 279-290.

Erik D. Milestones in the discovery of antiviral agents: nucleosides and nucleotides. Acta Pharmaceutica Sinica B.2012;6:535-548.

Erik D. New developments in anti-HIV chemotherapy. Biochimitaet Biophysica Acta .2002;1587:2-3: 258-275.

Ersan M. Number of HIV patients in Turkey up fourfold in last 10 years. Hurriyat Daily news, Oct 26, 2017.

Esposito F, Corona A, Tramontano E. HIV-1 Reverse Transcriptase Still Remains a New Drug Target: Structure, Function, Classical Inhibitors, and New Inhibitors with Innovative Mechanisms of Actions. Mol Biol Int. 2012;2012:586401.

Famiglini V, Silvestri R. Transcriptase Inhibitors. Molecules. 2016;21:221: doi:10.3390/molecules21020221.

Fellay J, Boubaker K, Ledergerber B et al., Swiss HIV Cohort Study. Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. Lancet.2001;358:1322-1327.

Geronikaki A, Eleftheriuo P, Poroikov V. Anti-HIV Agents: Current Status and Recent Trends. Topics in medicinal chemistry. 2016;1-59 Doi 10.1007/7355_2015_5001.

Gökengin D, Doroudi F, Tohme J, Collins B, Madani N. HIV/AIDS: trends in the Middle East and North Africa region. International Journal of Infectious Diseases.2016;44:66-73.

Gülümser Ç, Erbaydar T. HIV/AIDS epidemic in Turkey and use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. Turkish journal of obstetrics and gynecology.2015;12:4:192-198.

Hammer S M, Eron JJ Jr, Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P, Fischl M A, Gatell J M, Hirsch M S, Jacobsen D M, Montaner J S, Richman D D, Yeni P G, Volberding P A. Antiretroviral Treatment of Adult HIV Infection Recommendations of the International AIDS Society-USA panel. JAMA.2008;300:5:555-570.

Hemelaar J, Gouws E, Ghys PD, Osmanov S. WHO-UNAIDS Network for HIV Isolation and Characterisation.. Global trends in molecular epidemiology of HIV-1 during 2000 –2007. AIDS. 2011;25:679–689.

Hemelaar, J. The origin and diversity of the HIV-1 pandemic. Trends. Mol. Med.2011; 18:182–192.

Henrinch TJ and Kuritkes DR. HIV-1 entry inhibitors: recent development and clinical use. Current Opinion HIV AIDS.2013;3:1:51-57.

Hoenigl M, Chaillon A, Kessler HH, Haas B, Stelzl E, Weninger K et al. Characterization of HIV Transmission in South-East Austria. PLoS ONE.2016;11:3: e0151478. https://doi.org/10.1371/journal.pone.0151478.

Hoenigl M, Graff-Zivin J, Little SJ. Costs per Diagnosis of Acute HIV Infection in Community-based Screening Strategies: A Comparative Analysis of Four Screening Algorithms. Clin Infect Dis.2016;62:501–511.

Hoenigl M, Weibel N, Mehta SR, Anderson CM, Jenks J, Green N, et al. Development and Validation of the San Diego Early Test (SDET) Score to Predict Acute and Early HIV Infection Risk in Men who have Sex with Men. Clin Infect Dis.2015;61:468–475.

Hollingsworth TD, Anderson RM, Fraser C. HIV-1 Transmission, by Stage of Infection, The Journal of Infectious Diseases.2008;198:5:687–693

Imamichi T. Action of Anti-HIV Drugs and Resistance: Reverse Transcriptase Inhibitors and Protease Inhibitors. Current Pharmaceutical Design.2004;10:32:4039-4053.

Jacobs GB, Laten A, vanRensburg E, Bodem J, Weissbrich B, , Rethwilm A , Preiser, W, Engelbrecht S. Phylogenetic diversity and low level antiretroviral resistance mutations in HIV type 1 treatment-naïve patients from Cape Town, South Africa. AIDS Res Hum Retroviruses.2008;24:1009-1012.

Jacobs GB, Schuch A, Schied T, Preiser W, Rethwilm A, Wilkinson E, Engelbrecht S, Bodem J. Construction of a high titer Infectious and novel HIV-1 subtype C proviral clone from South Africa, pZAC. Submitted to J Gen Virol.2011.

Jialun, Z Sirisanthana T, Kiertiburanakul S. Trends in CD4 counts in HIV-infected patients with HIV viral load monitoring while on combination antiretroviral treatment: results from the TREAT Asia HIV Observational Database. BMC Infect Dis.2010;10:361.

Johnson VA, Brun-Vezinet F, Clotet B, Conway B, D'Aquila RT, Demeter LM, Kuritzkes DR, Pillay D, Schapiro JM et al. Drug resistance mutations in HIV-1. Top HIV Med.2003;11:215-221.

Kagan R, Winters M, Merigan T, Heseltine P. HIV type1 genotypic resistance in a clinical database correlates with antiretroviral utilization, AIDS Res HumRetroviruses.2004;20:1-9.

Kirchhoffe F. HIV life cycle :overview Encyclopedia of AIDS.2013;1-9.

Kuritzkes DR. HIV-1 entry inhibitors: an overview. Current Opinion HIV AIDS. 2009;24:82-87.

Laurent C, Kouanfack C, Vergne L, Tardy M, Zekeng L, Noumsi N, Butel C, Bourgeois A, Mpoudi-Ngolé E, Koulla-Shiro S, Peeters M, Delaporte E. Antiretroviral Drug Resistance and Routine Therapy, Cameroon. Emerging infectious diseases.2006;12:6:1001-1004.

Leal E, Villanova FE. Diversity of HIV-1 subtype B: implications to the origin of BF recombinants. PloS one.2010; 5:7: e11833. doi:10.1371/journal.pone.0011833.

Mataftsi M, Skoura L, Sakellari D. HIV infection and periodontal diseases: an overview of thepost-HAART era.2010;17:1:13-25.

Menendez-Arias L, Gago F.Antiviral Agents: Structural Basis of Action and rational design.2013;68:599-630.

Mumtaz GR, Weiss HA, Thomas SL, Riome S, Setayesh H, Riedner G, Semini I, Tawil O, Akala FA, Wilson D, Abu-Raddad LJ. HIV among People Who Inject Drugs in the Middle East and North Africa: Systematic Review and Data Synthesis.PLoS Medicine. 2014;1:6:doi:10.1371/journal.pmed.1001663.

Ndembi N, Hamers RL, Sigaloff KC et al. Transmitted antiretroviral drug resistance among newly HIV-1 diag nosed young individuals in Kampala. AIDS.2011;25:7:905-910.

Patrikar S, Basannar DR, Bhatti VK, Kotwal A, Gupta RM, Grewal RS. Rate of decline in CD4 count in HIV patients not on antiretroviral therapy. Medical journal, Armed Forces India.2013;70:2:134-138.

Phillips K D. Protease Inhibitors: A New Strategy Against HIV.JANAC.1996;7:5:57-71.

Ravichandran S, Veerasamy R, Raman S, Krishnan P N, Agrawal R K. An Overview On Hiv-1 Reverse Transcriptase Inhibitors. Digest Journal of Nanomaterials and Biostructures.2008;3:4:171-187.

Salzberger B, Marcus U, Vielhaber , Arasteh K, Gölz J, Brockmeyer NH, Rockstroh J. German-Austrian Recommendations For the Antiretroviral Therapy of HIV-Infection. European Journal Of Medical Research. 2004;9:491-504.

Sargin F and Goktas S. HIV prevalence among men who have sex with men in Istanbul. International Journal of Infectious Diseases.2017;54:58-61.

Schulter E, Oette M, Balduin M et al. HIV prevalence and route of transmission in Turkish immigrants living in North-Rhine Westphalia, Germany. Med. Microbiol. Immunol.2011;200:219–223.

Shafer RW. Genotypic testing for human immunodeficiency virus type 1 drug resistance. Clinical microbiology reviews.2002;15:2:247-77.

Shaw GM, Hunter E. HIV transmission. Cold Spring Harbor perspectives in medicine.2012; 2:11:a006965. doi:10.1101/cshperspect.a006965.

Sprinz E, Netto EM, Patelli M, Lima JS, Furtado JJ, da Eira M, Zajdenverg R, Madruga JV, Lewi DS, Machado AA, Pedro RJ, Soares MA. Primary antiretroviral drug resistance among HIV type 1-infected individuals in Brazil.AIDS Res Hum Retroviruses. 2009;25:9:861-867.

Strasfeld L, Chou S. Antiviral Drug Resistance: Mechanisms and Clinical Implications. Infect Dis Clin North Am.2010;24:2:413–437.

Taiwo B. Understanding transmitted HIV resistance through the experience in the USA. International Journal of infectious diseases.2009;13:5:552-559.

Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. N Engl J Med. 2008;358:15:1590-602.

Engelman A, Cherepanov P. The structural biology of HIV-1: mechanistic and therapeutic insights. Nat Rev Microbiol. 2012;10:4:279-290.

Thompson MA, Aberg J A, Cahn P, Montaner J S, Rizzardini G, Telenti A, Gatell JM, Günthard HF, Hammer SM et al. Antiretroviral treatment of adult HIV infection:

recommendations of the International AIDS Society-USA panel. JAMA.2010;304:3: 321-333.

Tsamis F, Gavrilov S, Kajumo F, Seibert C, Kuhmann S, Ketas T, Trkola A, Palani A, Clader JW, Tagat JR, et al. Analysis of the mechanism by which the small-molecule

CCR5 antagonists SCH-351125 and SCH-350581 inhibit human immunodeficiency virus type 1 entry. J Virol.2003;77:5201-5208.

U.S. Food and Drug Administration. Antiretroviral drugs used in the treatment of HIV infection. www.fda.gov. (assessed June 7, 2018).

Vercauteren J, Vandamme AM. Algorithms for the interpretation of HIV-1 genotypic drug resistance information, Antiviral Res.2006;71(2-3):335-342.

Vercauteren J, Wensing AM, Van de Vijver DA et al. Transmission of drug-resistant HIV-1 is stabilizing in Europe. J. Infect. Dis.2009;200:10:1503-1508.

Viani RM, Peralta L, Aldrovandi G, Kapogiannis BG, Mitchell R, Spector SA, Lie YS, Weidler JM, Bates MP, Liu N. Prevalence of Primary HIV-1 Drug Resistance among Recently Infected Adolescents: A Multicentre Adolescent Medicine Trials Network for HIV/AIDS Interventions Study . The Journal of Infectious Diseases.2006;194:11:1505-1509.

Watson C, Jenkinson S, Kazmierski W, Kenakin T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric non competitive ÖHIV entry inhibitor. MolPharmacol.2005;67:4:1268-1282.

WHO (2018) HIV/AIDS Global health sector strategy on HIV, 2016-2021.

WHO Factsheet (2017)WHO commends UNITAID for new investment in advanced HIV disease, hepatitis, June 2017.