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Human Immuno-Deficiency Virus Drug Resistance, Nucleoside Reverse Transcriptase Inhibitors, Non-nucleoside Reverse Transcriptase Inhibitors and associated drug resistance mutations in the Reverse Transcriptase Gene of Human Immuno Deficiency Virus-1.

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Corresponding Author:
Dr. Keshar Mohanty,
Scientist, National JALMA Institute for Leprosy and Other Mycbacterial Diseases, TajganJ, 282001 - India

Submitting Author:
Mr. Sushanta K Barik,
Research Scholar, National JALMA Institute for Leprosy and Other Mycbacterial Diseases, TajganJ, 282001 - India

Other Authors:
Dr. Srikanth Tripathy,
Scientist, National Institute for Research in Tuberculosis, Chennai, 600031 - India

Dr. Umesh Gupta,
Scientist, National JALMA Institute for Leprosy and Other Mycbacterial Diseases, TajganJ, Agra, 282001 - India

Dr. Deepa Bisht,
Scientist, National JALMA Institute for Leprosy and Other Mycbacterial Diseases, TajganJ,Agra, 282001 - India

Dr. Srikanta Jena,
Lecturer, Ravenshaw University, Cuttack,Odisha, 753003 - India

Dr. Partha Mohanty,
Scientist, National JALMA Institute for Leprosy and Other Mycbacterial Diseases, Tajg-Ganj, Agra, 282001 - India

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Human Immuno-Deficiency Virus Drug Resistance, Nuclesoide Reverse Transcriptase Inhibitors, Non-nuclesoide Reverse Transcriptase Inhibitors and associated drug resistance mutations in the Reverse Transcriptase Gene of Human Immuno Deficiency Virus-1.

Author(s): Barik SK, Mohanty K, Tripathy S, Gupta U, Bisht D, Jena S, Mohanty P

Abstract

Resistance develops due to mutations in the reverse transcriptase gene of HIV-1 genome that does not respond towards the presence of effective drugs. Emergence of HIV-1 variants in the presence of effective drugs is a common occurrence. Drug resistance mutation means “the development of resistance mutations in the drug targeted HIV-1 genes” which causes the viruses to overcome the drug pressure. Thus resulting in failure to antiretroviral therapy. The detection of these mutations is possible by genotypic assay and the analysis of the effect of different drug concentrations towards the reverse transcriptase gene is possible by phenotypic assay. Genotyping of these mutations gives the information on therapeutic decision at the population and individual level. The frequency of mutation in the HIV-1 genes varies in the same population of a region or a city. The frequencies of mutations in the reverse transcriptase genes of HIV-1 lead to subtype diversity, drug resistance mutations and formation of recombinant isolates. The frequency of mutations in the reverse transcriptase gene is a cause of treatment failure by nucleoside/ nucleotide reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors in a population level. The frequency of mutations in the reverse transcriptase gene is directly related to the prevalence of HIV-1 drug resistance mutations in a population or individual level.

This review mainly focuses on the human immunodeficiency virus drug resistance, nucleoside/nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and associated drug resistance mutations in the reverse transcriptase gene of human immuno-deficiency virus-1.

Abbreviations:

HIV: Human Immunodeficiency Virus, AIDS:Aquired Immunodeficiency Syndrome. HIVDR: Human Immuno deficiency virus drug resistance, NRTIs:Nucleoside reverse transcriptase inhibitors, NNRTIs:Non-nucleoside reverse transcriptase inhibitors, RT: Reverse transcriptase, PIs:Protease inhibitors, TAMs: Thymidine analogous mutations, NonTAMs: Non-thymidine analogous mutations.

Introduction

Acquired Immuno-Deficiency Syndrome in human beings is caused by two viruses Human Immunodeficiency Virus -1(HIV-1) and Human Immunodeficiency Virus-2 (HIV-2) in which the HIV-1 is more virulent than HIV-2.This disease was first reported in some patients of USA in the year 1981 [1] **.** HIV-1 groups were classified as group M, group O, group P and a new group N (Non M/Non O). HIV-1 group M pandemic spread in Kinshasa around early 1920s and the epidemic histories of HIV-1 group M and group O were similar until 1960s, after which group M under gone epidemiological transition through social changes and transport networks and established in human population[2]. The Global burden of HIV/AIDS is directly related with the disability adjusted life years of the individuals [3]. The HIV RNA is ≥ 9 kb, a single stranded RNA consisting of 15 proteins [4]. Human Immuno-Deficiency Virus (HIV) is transmitted by sexual contact, blood transfusion, blood semen, vaginal secretion and breast milk as well as oral sex. During the pregnancy, the HIV is transmitted from mother to the baby or breast feeding. HIV is not transmitted through saliva and casual contact such as kissing or sharing glasses. The first infection of HIV is called primary infection like severe flu, headache, fever, throat, rash, swollen glands, headache, ulcers in mouth [5].It is universally accepted that the highly active antiretroviral therapy

(HAART) has reduced the HIV related mortality worldwide. The implementation of ART programmes reduces the morbidity and mortality in the population level. The scaling up of the ART may be controlled the drug resistance mutations and transmission of HIV drug resistance strains in a given population. The combination of antiretroviral therapy regimens were developed in the late 1990s. This therapy is used to suppress the viral replication and decrease the disease progressiveness. India is a developing nation where it required the rapid scale up antiretroviral therapy to decline the mortality and morbidity in the population level [6] .

The six drug classes are 1.Nucleoside reverse transcriptase inhibitors 2.Non-nucleoside reverse transcriptase inhibitors 3.Integrase inhibitors 4.Protease inhibitors 5.Fusion inhibitors 6.Coreceptor antagonists. Now a days in many hospitals, the most significant management in HIV infection has been treated by the antiviral drugs. The antiviral drugs suppress the viral replication up to undetectable levels. Now Food and Drug Administration, USA approved twenty five drugs under this six classes drug categories which are available for treatment of HIV-1 infection [7].

Now a days, the most significant way of management in HIV infection is the treatment with antiviral drugs in majority of health settings. At present, the first line treatment for HIV consists of two nucleoside reverse transcriptase inhibitors (NRTI's), a thymidine analog zidovudine , stavudine or lamivudine and one non-nucleoside reverse transcriptase inhibitor (NNRTI), a cytidine analog nevirapine or efavirenz. The protease inhibitors (PIs) are available for second line treatment options upon failure of first line treatment under national programme. The drug resistance in HIV is defined as "Identification of any drug resistance mutation in human immunodeficiency virus in patients during the combined antiretroviral therapy. The drug resistance mutation recorded the virologic failure of the patients and the date of virologic failure as per the 2010 WHO guidelines on antiretroviral therapy in adults and adolescents defined as the first recorded date of the plasma viral load of more than 5000 copies /ML after six months of treatment. According to WHO protocol $CD4^+$ cells should be < 350 cells / µl in decreasing order followed by HAART is consider as HIV drug resistance [8].

The genetic diversity of HIV-1 is extensive by comparing the data from Los Alamos HIV Sequence Data base, HIV db drug resistance interpretation algorithm (version 4.1.9), ANRS drug resistance interpretation algorithm (version 2005.07) and Rega institute drug resistance interpretation algorithm (version 6.4.1), IAS-USA available as online tool[9].

HIV Drug Resistance (HIVDR)

The ability of HIV to mutate and reproduce in the presence of antiretroviral drugs is called HIV drug resistance. The viral replicates and survives in the presence of inhibitors like nucleoside/nucleotide inhibitors, non-nucleoside inhibitors, protease inhibitors etc.

Based on the population level HIVDR is divided in to three types 1.Transmitted HIVDR 2 .Acquired HIVDR 3. Pre-treatment HIVDR

1. Transmitted HIV Drugresistance:

Transmission of the drug resistant variants in to newly infected individuals is termed as transmitted drug resistance. The transmitted drug resistance is directly related to ART service, duration as well as fitness of the drug resistance strains that evolve. Transmitted HIVDR is observed in some populations those are unexposed to antiretroviral therapy. After initial infection, the certain drug resistance mutations rapidly changes in to wildtype due to without drug selective pressure. In some cases exposure of antiretroviral therapy leads to revert the mutations leading to virologic failure. Transmitted HIVDR strains are transferred from one patient to another. The prevalence and incidence rate of HIV-1 was high in the fishing communities on the Lake Victoria in Uganda .Among the population the spread of HIV-1 epidemic is due to transmitted drug resistance (TDR). The TDR data were collected from the Uganda population among the antiretroviral naive and some recently infected individuals about five years after antiretroviral scaling- up. The NNRTIs drug resistance mutation K103N was identified in two individuals and V106A in one individual in naive population of Uganda. It was suggested that the level of TDR was moderate in this population of Uganda. No NRTIs or PIs drug resistance mutations were detected in Uganda population [10].

The HIV prevalence is rising among the men sex with men (MSM) group of population in china. The MSM group were married due to social pressure and therefore, they may have the wives and sexual partners. So the group with transmitted drug resistance have a risk of transmitting the HIV subtypes to both their male and female sexual partners [11].The transmitted drug resistance is an important contribution of local transmission clusters and the spread of resistant virus in the MSM group of Croatia

[12].The presence of transmitted drug resistance in treatment naive HIV-1 infected patients gradually increases in the countries like Soviet union where the antiretroviral therapy is exposed [13].

2. Acquired HIVDrugresistance:

This kind of HIVDR emerges in response to drug selective pressure by different drug classes. In case of HIVDR the optimal regimens are provided as well as adherence is supported. This kind of drug resistance mutation develops due to infection with a second strain called as "super infection". A case was reported in HIV-1 super infection with a subtype- B, successfully controlling in viremia with continuous combination of triple class antiretroviral therapy started 8 years earlier during primary HIV infection [14].

3. Pre-treatment HIVDrugresistance:

Pre-treatment HIVDR is emerged in populations initiating antiretroviral therapy for the first time. The pre-treatment HIVDR emerge due to antiretroviral drug exposure during the earliest infection time, the prevention of mother to child transmission, pre exposure prophylaxis, post-exposure prophylaxis. Sometimes poor adherence to ART in a population leads to the drug resistance mutation. A study was reported in Northwest Spain in the high prevalence subtype F , the non-B subtype among newly diagnosed HIV-1 infected persons.The impaired treatment response to ART leads to slower virologic response in Men Sex with Men (MSM) group of population in Northwest Spain [15]. The prevention of mother to child transmission of HIV is essential by use of suitable regimens. In a population study in five centres of Johannesberg, the treatment naive infected children's were developed drug resistance mutations towards non-nucleoside reverse transcriptase inhibitors.The Y181C confers high level resistance to nevirapine exposed childrens but was confers intermediate level resistance to efavirenz and etravirine. Although the use of efavirenz in children under three years of age is controversial but the use of lopinavir/ritonavir regimen towards newly diagnosed HIV infected childrens in the era of more efficacious antiretroviral prophylaxis [16].

HIV-1 reverse transcriptase

The reverse transcriptase enzyme is responsible for RNA dependent DNA polymerization as well as DNA dependent DNA polymerization. Reverse transcriptase is a heterodimer consisting of two subunits like p66 and p51. The p66 subunit is composed of 560 amino acids and p51 subunit is composed of 450 amino acids. The p66 subunit is active contains the DNA binding groove and active site ,the p51 subunit has no enzymatic activity .The p66 subunits has five domains like fingers, palm, thumb sub- domains which participate in polymerization and the other domains connection and RNase H sub-domains .The nucleoside reverse transcriptase inhibitors are triphosphorylated by the action of host cellular enzymes. The triphosphorylated nucleoside triposphates incorporated in to the newly synthesiz deoxyribonucleotides to cause chain termination [17].

Nucleotide/Nucleoside reverse transcriptase inhibitors (NRTIs):

Nucleoside reverse transcriptase inhibitors are the main drug used to treatment of both naive and experienced HIV-1 infected individuals. NRTIs can be divided in to two broad categories like Thymidine analogues mutations (TAMs) and Non-thymidine analogous mutations (Non-TAMs).

The examples of the TAMS are zidovudine (3'Azido 2'3' di-deoxy guanosine) and Stavudine (2'3'-didehydro 3'deoxythymidine). The examples of Non TAMs are didanosine (2'3'dideoxyinosine),zalcitabine(2'3'dideoxycitidine),lamivudine(2'deoxy3'thiothiadine),emtricitabine(-)B-1-3'-thio-2'-3'-dideoxy-5flurocytidine,FTC,abacavir(IS,4R)-4-[2-amino-6 (cyclopropylamino)-9H-purin-9-y1]-2cyclo-pentene-1-methanolsuccinate),(ABC) [18].

General features of nucleoside reverse transcriptase inhibitors (NRTIs)

General features of thymidine analogus mutation drugs (TAMs):

Zidovudine:

Zidovudine was the first drug synthesized by Horwitz at the cancer Michigan foundation in the year 1964. This drug has the retroviral activity. In the year 1986, the drug has provided the strong evidence that zidovudine prolongs survival, decrease the severity of infections and improve the quality of life those are infected with HIV/AIDS [19] .Then in the year 1987, zidovudine was first approved drug by food and drug administration. Zidovudine inhibits the activity of

reverse transcriptase of HIV-1 but not the activity of human DNA polymerase. The minimum dosage of therapeutic requirement for HIV-1 treatment is 300mg/ twice a day. Zidovudine has several risks in the body of human being causes bone marrow suppression, headache, fatigue, neutropenia, nausea, vomiting, hematological toxicity, myalgia, anaemia, neutropenia, myopathy and lactic acidosis with hepatosteatosis is a rare but life threatening mitochondrial toxicity [20].

Stavudine:

Stavudine (2'3'-didehydro 3'deoxythymidine) was synthesized by Jerome Horwitz in the year 1960. It was an HIV agent declared by Rega Institute for Medical Research in Belgium [21]. It was then approved by USA food and drug administration in the 24th June 1994. Stavudine inhibits the activity of reverse transcriptase of HIV-1 but not inhibit the activity of DNA polymerase. Because of its irreversible side effects, less toxic.Stavudine is used as a first line therapy in developing countries. Stavudine is not metabolized by cytochrome P450. The stavudine is mainly used in opportunistic infections in the human immuno-deficiency virus infected patients [22]. The minimum 30 mg to 40 mg therapeutic dosages required for HIV infected patients. The stavudine induced the multiple adverse effects in HIV infected patients like hepatic steatosis, hyper lactatemia, lipodystrophy, peripheral neuropathy, dyslipidemia, hyperglycemia and mitochondrial dysfunction [23].

General features of the non thymidine analogous mutation (Non TAMs) drugs:

Didanosine:

The drug didanosine was initially synthesized by Morris J.Robins and R.K. Robins in the year 1964 [24]. Didanosine became the second USA approved drug by FDA in the year 9th October 1991.The most common effect of didanosine are diarrhea, nausea, vomiting, abdominal pain, fever, headache and rash [25]. Didanosine is a nucleoside analogue of (2'3' dideoxyinosine) guanosine. It inhibits reverse transcriptase by competing with natural dATP but donot inhibit the activity of DNA polymerase. From the pharmacokinetic study, it is known that, the didanosine is an acid labile anti retroviral agent. The minimum therapeutic dosage is 375mg/day required for treatment of HIV infected patients [26].

Zalcitabine:

Zalcitabine (2′-3′-dideoxycytidine, ddC) was synthesized by Jerome Horwitz and subsequently declared as an anti HIV agent by Samuel Broder, Hiroaki Mitsuya and Robert Yarchoan at the National Cancer Institute [27]. Zalcitabine was approved by Food and Drug Administration on 19th June 1992. Zalcitabine significantly inhibits the intracellular phosphorylation, then convert in to active form. So the drugs are not administered together. Zalcitabine phosphrylated in T cells and other HIV target cells in to active triphosphate forms.This active triphosphate work as a substrate for HIV reverse transcriptase and also incorporating in to the viral DNA. Hence, terminating the chain elongation.The most common adverse effects on human being is nausea, headache, peripheral neuropathy, oral ulcers, peripheral ulcers etc [28].

Lamivudine:

Lamivuidne (2′, 3′-dideoxy-3′-thiacytidine) was invented by Dr Bernard Belleau in the Mc Gill University and Dr Paul N guyen-Ba at the Monetral based IAF Biochem International incorporation laboratories in the year 1988 and 1989.Dr Yung-Chi Cheng of Yale University reported that lamivudine work well when combine with zidovudine to inhibit the activity of reverse transcriptase .The 300 mg/day is the minimum dose used by the HIV infected patients with zidovudine. Lamivudine has no carcinogenicity or mutagenicity but as less toxic to mitochondrial DNA adverse effects like skin rash has been reported in HIV infected patients those are taking antiretroviral therapy [29, 30].

Emtricitabine:

Emtricitabine is discovered by Dr Dennis C.Liotta, Dr Raymond F. Schinazi and Dr Woo -Baeg Choi, Emory University and immediately licensed by Triangle pharmaceuticals in the year 1996. It was approved by FDA in the 2nd July 2003. Emtricitabine is an analogue of cytidine. Emtricitabine acting well by inhibiting the activity of reverse transcriptase .HIV lower the amount of viral load in the patient body that can increase the number of CD4 cells in the immune system. The treatment of toxicity with Emtricitabine is unusual .The treatment related adverse effects are diarrhea, nausea, vomiting and rash. The severe side effects may experience hepatotoxicity or lactic acidosis. The minimum 600mg/day in combination therapeutic doses required for treatment of HIV infected patients [31].

Abacavir:

Abacavir is an anti-retroviral drug inhibit the reverse transcriptase used to treat HIV .The main side effect of the drug is hypersensitivity which can be severe. The other side effects are vomiting, diarrhea, nausea etc. Abacavir was approved by FDA on December 18 in the year 1998**.**The minimum 600mg/day therapeutic doses is required to treat HIV infected patients [32].

Mechanism of chain termination by the nucleoside reverse transcriptase inhibitors (NRTIs) :

Nucleoside reverse transcriptase inhibitors were the first drugs to approve by Food and Drug Administration, USA. Before antiviral effects, the nucleoside reverse transcriptase inhibitors entry in to cells, the phosphorylation occurs by cellular kinases. The absence of 3' hydroxyl group at the sugar moiety of the nucleoside reverse transcriptase inhibitors prevents the formation of phosphodiester bonds at the 3'-5' end that prevents the bond formation between incoming nucleoside triphosphates (5'end) and nucleoside reverse transcriptase inhibitors. This chain termination event occurs in RNA dependent DNA synthesis or DNA dependent DNA synthesis during the production of proviral deoxyribonucleotides [33].

Mechanism of emergence of drug resistance:

Nucleoside reverse transcriptase inhibitors mutations are occurred in reverse transcriptase and classified as Nucleoside associated mutations or Thymidine analogous mutations. Genetic variations are observed in HIV-1 due to drug selective pressure among the treated and untreated patients those are taking antiretroviral therapy. The emergence of drug resistance mutations is the cause of drug susceptibility towards the HIV strains.

Two types of mechanism are behind for the resistance to nucleoside reverse transcriptase inhibitors. 1. ATP dependent pyrophosphorylysis 2.Prevention of NRTIs in to nascent chain.

1. ATP dependent pyrophosphorylysis: Removal of NRTIs from the 3´end of the nascent chain and chain termination in a reversal way as well as the increased the discrimination between the dNTPs and inhibitors.Thymidine analogous mutations (TAMs) promotes pyrophosphorolysis and involved in the excision of zidovudine (AZT) and stavudine (d4T) [34,35] . Thymidine analogous mutation amino acid changes (TAMs) in HIV-1 reverse transcriptase includes two phases TAM1 and TAM2 phases. The TAM1 pathway amino acid changes includes (T215Y, L210W, D67N M41L) and the TAM 2 pathways amino acid changes includes (D67N, K70R, T215F and 219E/Q) [36,37,38,39,40].

2. Prevention of NRTIs in to nascent chain: Some mutations emerge in the reverse transcriptase region which prevent the interaction with nucleoside reverse transcriptase inhibitors. The mutations M 184V, K65R emerge as a resistant in the reverse transcriptase region those patients are taking zidovudine, lamivudine, emtricitabine, abcavir. This mutation decreases the reverse transcriptase function and replicative fitness of the virus[41,42].

Non-nucleoside reverse transcriptase inhibitors (NNRTIs):

Four non-nucleoside reverse transcriptase inhibitors are presently used by HIV infected patients. The nevirapine, efavirenz, etravirine, rilipivirne are commonly used by HIV infected patients in ART clinic. Based on the resistance activity, two generations of NNRTIs were developed. In first generation NNRTIs nevirapine (NVP), delavirdine (DLV) and efavirenz are drugs with low genetic barrier and poor resistance profile which has led to development of second generation NNRTIs. Etravirine (ETR) and rilpivirine has been developed by Food and Drug Administration as well as European union.

The next generations of NNRTIs is being clinically developed : Lersivirin , MK-1439, MK-6186 GSK 2248761, RDEA806, BILR 355 BS, Calanolide A, MK-4965 and their pharmacokinetics, metabolism, clinical data, safety and tolerability is now on the way of testing and research [43].

General features of first generation Non-nucleoside reverse transcriptase inhibitors (NNRTIs):

The first NNRTIs to be discovered are 1-(2-2-hydroxyethoxymethyl)-6-(phenylthio) thymine (HEPT) and tetra hydroimidazo [4,5,1-jkj] [1,4] benzodiazepin-2(1H)-one and-thione (TIBO) are specific for HIV-1 inhibitors. Both HEPT and TIBO are highly active compounds against HIV-1 but they are inactive against HIV-2. After HEPT and TIBO, several other compounds were discovered and later on FDA approved as NNRTIs including dipyridodiaze pinones (NVP),bis(heteroaryl) piperazines (DLV) and benzoxazinones(EFV) [44].

Nevirapine :

Nevirapine is a dipyridodiazepinone (11-cyclopropyl-5, 11-dihydro- 4-methyl-6H-dipyrido [3, 2-b: 2', 3'-e] [1,4] diazepin- 6- ranges from 200 mg once daily up to 14 days followed by 200 mg twice daily. In humans, nevirapine undergoes hepatic biotransformation to several hydroxylated metabolites (2-, 3-, 8- and 12-hydroxyl nevirapine) by CYP which is also an auto-inducer of isoenzymes 3A4 (CYP3A4) and 2B6 (CYP2B6). Nevirapine is a noncompetitive one). Nevirapine is a low water soluble and lipophilic drug. Beacuse of its weak basic character and ionization, nevirapine exhibits pH dependent solubility .Nevirapine is highly soluble in buffer. The nevirapine is a class-II compound because of its low solubility and high intestinal permeability [45]. Effective dosage inhibitor of HIV-1 reverse transcriptase but does not

have a significant inhibitory effect on HIV-2 RT or eukaryotic DNA polymerase α, β, γ, δ. Nevirapine has been given to the pregnant women .The main role of nevirapine is to prevent mother to child transmission of HIV-1 infection [46,47].

Efavirenz:

Efavirenz (EFV) is a benzoxazinone derivative (S)-6-chloro-4 (cyclopropylethynyl) -1,4-dihydro-4-(tri-fluoromethyl)-2H-3,1-benzoxazin-2-one). EFV is a crystalline non-hygroscopic lipophilic materials, class-II drug has the characteristics properties of low solubility and high permeability. In vitro studies demonstrated that Efavirenz is metabolized in the liver by the CYP3A4 and CYP2B6 isoenzymes[48]. Effective doses of 200-400 mg daily required for 10 days for the HIV infected patients. Efavirenz is a non-competitive inhibitor of HIV-1 RT but does not have a significant inhibitory effect on HIV-2 reverse transcriptase or on human DNA polymerase α β γ or δ. The mechanism of viral resistance or reduced susceptibility depends upon the mutations of HIV-1 reverse transcriptase [49].

Delavirdine:

Delavirdine (DLV) belongs to a bisheteroarylpiperazines derivative (N-[2-({4-[3-(propan-2-yl amino) pyridin-2-yl] piperazin-1-yl} carbonyl)-1H-indol-5-yl] methanesulfonamide).Delavirdine is a weak base and no data is available about BCS Class compounds. The DLV therapeutic dose is 400 mg three times a day.The drug is highly protein bound in humans is metabolized by CYP3A4 into biologically inactive metabolites: N-dealkylated metabolite and unidentified pyridine hydroxy metabolites: N-dealkylated metabolite and unidentified pyridine hydroxy metabolites (including Met-7 and Met-7a [50]. Delavirdine is highly selective for HIV-1 reverse transcriptase and it has minimal effects against HIV-2 reverse transcriptase as well as human cellular DNA polymerase α and β .Delavirdine has been assigned to the pregnancy category C by FDA and have no effectiveness has been reported related to age or sex[51] .

Second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs):

The second generations NNRTIs include etravirine and rilpivirine which belongs to the family of di-aryl-pyrimidine compounds. This kind of NNRTIs shown better resistance profile.

Etravirine:

Etravirine has been better used in treatment experienced multidrug resistant HIV-1 infected adults where as rilpivirine has been used to treat naïve -treatment HIV-1 infected patients [52].The etravirine a lipophilic drug has low solubility, permeability properties and categorized under IV class compounds [53].The therapeutic dosages of etravirine is 200 mg twice daily.The etravirine is primarily metabolized by several CYP isozymes like CYP3A, CYP2C9 and CYP2C19[54].

Rilpivirine:

Rilpivirin is a (4-[[4-[4-[(E)-2-cyanoethenyl]-2,6-dimethylanilino]pyrimidin-2-yl] amino] benzonitrile) compound and very poor water soluble compound. Rilpivirine is a class -II compounds with the properties of low solubility and high permeability. Rilpivirine is mainly metabolized by the CYP3A isoenzyme system. Rilpivirine activity is mediated by non competitive inhibition of HIV-1 RT and does not inhibit cellular DNA polymerases like δ, β, γ. The 25 mg of therapeutic dosages of rilpivirine is daily required by the HIV-1 infected patients [55].

Mechanism of action of non-nucleoside reverse transcriptase inhibitors:

Non-nucleoside reverse transcriptase is non-competitive inhibitors binding to a hydrophobic pocket in the HIV-1 RT near polymerase active site to stop the polymerization. NNRTIs do not interfere with substrate binding but interfere with the dNTPs binding.

Drug resistance mutations in HIV-1 reverse transcriptase gene:

The highly active antiretroviral therapy (HAART) includes the two nucleotide /nucleoside reverse transcriptase inhibitors (NRTIs) with one non-nucleoside reverse transcriptase inhibitors (NNRTIs) or one protease inhibitor (PI) or one integrase inhibitor (II). The highly active antiretroviral therapy includes a single tablet regimen used by HIV patients. Resistance mutations development in the reverse transcriptase gene is a cause of treatment failure by the highly active antiretroviral therapy. A single tablet regimen is always associated with viral suppression and higher adherence than multiple tablet regimens in homeless and household AIDS patients [56]. Many times a single tablet regimen is not effective against drug resistance patients. Then they have to change the different combination regimens to treat drug resistance patients. A single tablet regimen tenfovir, emtricitabine and efavirenz is not effective towards the treatment due to the persistence of K65R, K103N and M184V/I reverse transcriptase mutations in HIV-1 infected patients leads to virological failure[57].

Nevirapine which is a highly lipophilic drug shown drug susceptibility in cell culture. Genotypic analysis in the RT region has shown the mutations in the Y181C or V106A depending on the virus strain and type of cell lines. The most common Y 181 C resistance-associated mutations have been observed in vivo but substitutions at positions 103, 106, 108, 181, 188 and 190 have been also observed in the RT region. The K103N mutation is the most efavirenz random access mutations in the RT region of HIV-1 where as the other efavirenz mutations at positions 100, 106, 108, 181, 188, 190 and 225 affect the amino acid positions in the RT gene of HIV-1 [58,59]. The most random access Mutations found in the delavirdine resistant virus occurs at codon positions 103 and 181[60]. Rilpivirine resistance associated mutations like K101E, K101P, E138A, E138G, E138K,E138Q, V179L, Y181C, Y181I ,Y181V, H221Y, F227C, M230L, K101E/M184I were reported[61] .Rilpivirane is mainly effective towards the drug resistance mutations K103N generated by first generation NNRTIs. The sixteen mutations are associated with resistance to rilpivirine are K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C and M230I/L.The risk of virological failure in HIV-1 infected patients is due to the above mutations. The most common resistance mutations that emerge against rilpivirine is E138K[62].

The drug resistance mutations developed in slower rate in the presence of didanosine. The most common resistance associated mutations is L74V observed in vivo in the viral pol gene which confers cross resistance to zalcitabine. The other mutations observed include K65R and M184V in the presence of didanosine. The T69D is the most common zalcitabine drug resistance associated mutation observed in vivo. The zalcitabine resistance mutations at positions 65, 74, 75, 184 and 215 in the pol gene were observed rarely without any cross resistance to other NRTIs[63].The didanosine, zidovudine and zalcitabine was tested among the people with advanced HIV disease . The survival rate was longer in patients those were switched over zidovudine to zalcitabine but no longer survival among patients who were treated with didanosine[64].

The reverse transcriptase mutations responsible for the most nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors.The Two novel mutations Q145M/L have been reported to cause high level resistance to multiple nucleoside reverse transcriptase inhibitors i.e. zidovudine, lamivudine, stavudine, didanosine, tenfovir and abacavir as well as both non-nucleoside reverse transcriptase inhibitors i.e. nevirapine and efavirenz in both cell culture and enzymatic assays[65].Sometimes, a rare mutation in the HIV-1 reverse transcriptase cause drug resistance mutation, which confer high level resistance to the non-nucleoside reverse transcriptase inhibitors. A mutation I 132M which confers high level resistance to the non-nucleoside reverse transcriptase inhibitors nevirapine and delavirdine but is hyper-susceptibility nucleoside reverse transcriptase inhibitors lamivudine and tenfovir [66]. Mutation in the Reverse transcriptase of HIV-1 have a substantial impact on drug resistance phenotype and replication capacity. The introduction of mutation in the M184V in the reverse transcriptase of HIV-1 has a reduced susceptibility to abacavir, lamivudine and didanosine but increased susceptibility to zidovudine, stavudine and tenofovir. The replication capacity of the virus also decrease in the presence of this M184V mutation.

Similarly L74 V mutation in the reverse transcriptase of HIV-1 decreased the susceptibility to abacavir, lamivudine and didanosine but increased susceptibility to zidovudine and tenfovir. K101E and G190S decrease susceptibility to all nucleoside reverse transcriptase inhibitors but the K103N mutation had little effect on the susceptibility towards the nucleoside reverse transcriptase inhibitors. In some strains K101E, G190S, L74V mutation increased the replication capacity in the presence of non-nucleoside reverse transcriptase inhibitors but has the decreased replication capacity in the absence of non-nucleoside reverse transcriptase inhibitors [67]. A single mutation T215S persist in MSM group treatment naive population in Croatia and this mutation persists in a cluster of population those are taking NRTIs [68]. The monitoring and drug resistance testing is essential before initiation of antiretroviral therapy are recommended to facilitate the selection of appropriate ART. The HIV-1 drug resistance mutations were characterized among the drug naive HIV-1 infected patients and the reverse transcriptase inhibitor mutations A62V, K103N, Y181C were found in Northern Vietnam population[69].

Resistance to antiretroviral therapy is associated with mortality. The risk of emerging resistance mutations to first line therapy is important for both resource rich settings and resource poor settings. The patients with thymidine associated mutations confers high level resistance to zidovudine and stavudine.The patients with K65R mutation is associated with multiple nucleoside reverse transcriptase inhibitors like tenfovir, abacavir and didanosine [70].The detection of mutations in early virologic failure in HIV-1 infected

patients followed by antiretroviral therapy in the treatment naive patients is very critical.Such mutations are undetectable at baseline by population sequencing. The key mutations K65R, K103N,Y181C, M184V and M184I developed against lamivudine, tenfovir, nevirapine and efavirenz in the reverse transcriptase region were quantified by allele specific chain reaction [71].Structural, biochemical and genetic analysis showed that some group of non-nucleoside inhibitors bind to the hydrophobic pocket near the polymerase active site. Mutations in the hydrophobic sites of the amino acids in the reverse transcriptase region developed the drug resistance mutations towards the non-nucleoside reverse transcriptase inhibitors. The hydrophobic amino acid pocket like tyrosine 181, tyrosine 188 or lysine 103 leads to resistant to the non-nucleoside reverse transcriptase inhibitors [72].The HIV-1 pol gene diversity among the Brazilian patients those were taking nucleoside and non-nucleoside reverse transcriptase is the cause of drug resistance mutation in the reverse transcriptase region. The similar kind of reverse transcriptase nucleoside resistance mutations M41L (52%), D67N (30%), K70R (26%), M184V (88%), L210W (29%), T215Y/I/F (65%) and K219Q/E/N (28%) and reverse transcriptase non-nucleoside resistance mutation K103N (52%) were developed in all HIV-1 subtypes B, F and recombinant B/F viruses of Brazilian population[73].

In some patients, a single emergence of non-nucleoside reverse transcriptase associated drug resistance mutations in the reverse transcriptase region is a cause of treatment failure even the use of nucleoside reverse transcriptase inhibitors unable to prevent the emergence of the particular single mutation. The Y181C mutation in the reverse transcriptase region confers high level resistance mutations towards the non-nucleoside reverse transcriptase inhibitors but did not reduce or emerge the prevention of this Y181C mutation towards the nucleoside reverse transcriptase inhibitors [74]. The presence of base line mutations in the reverse transcriptase region associated with hyper susceptibility to non-nucleoside reverse transcriptase inhibitors.The base line mutations M41L, M184V, L210W and T215Y in the reverse .transcriptase gene is the cause of decrease viral load up to undetectable level [75]. The presence of the resistance associated mutations towards the first generation non-nucleoside reverse transcriptase inhibitors affect the replication kinetics of the virus and reduced the drug susceptibility even treated with second generation non-nucleoside reverse transcriptase inhibitors.In some patients K103N, M230L mutations in the reverse transcriptase gene containing viruses were severely impaired in replication capacity in the absence of NNRTIs. The K103N mutation containing viruses replicates well in the presence of efavirenz and nevirapine but the M230L mutation in the reverse transcriptase gene displayed substantial replication in the presence of all NNRTIs. The mutations K103N and M230L in the reverse transcriptase gene is associated with high level resistance to first generation NNRTIs efavirenz and nevirapine while the K103N containing viruses remains susceptible to second generation NNRTIs etravirine [76].The β3-β4 region in the reverse transcriptase gene of HIV-1 is associated drug resistance mutation. The point mutation, deletion and insertion in this region leads to development of drug resistance in the patients failing nucleoside reverse transcriptase therapy. The deletion or insertion in the Q151M site of β3-β4 region is associated with high level resistance towards NRTIs [77].The acquisition of thymidine associated mutations in the reverse transcriptase gene may lead to drug resistance towards zidovudine or didanosine. The N348I mutation in the HIV-1 RT associated with drug resistance mutation towards the other TAMs K70R, Y181C, L74V, M184V[78]. HIV-1 reverse transcriptase mutations in the connection sub-domain (CN) and RNaseH domain of HIV-1 reverse transcriptase of the subtype -B. HIV-1 infected patients enhance the nucleoside and non-nucleoside reverse transcriptase.

The mutations A376S, A400T, Q334D, G335D, N348I and A371V in the CN domain is associated with increased AZT resistance in the presence of thymidine analog mutations. The mutations L100I/ K103N/ Y181C in the CN domain enhanced the etravirine resistance in the treatment experienced Brazilian subtype C infected patients [79]. The 69 position deletion in the reverse transcriptase gene of HIV-1 confer high level resistance to nevirapine[80]. Etravirine is active against the drug resistance mutations K103N and Y181C in the RT region of HIV-1 [81].The non-nucleoside reverse transcriptase inhibitors etravirine and rilpivirine is conferred by E138K mutation in reverse transcriptase gene of the HIV-1. The mutations E138K/ M184I confer high level resistance to lamivudine and emtricitabine resistance in most patients with virologic failure. The replication capacity of the E138K/M184I mutant in the presence of etravirine was significantly higher than the E138K andE138K/M184V mutants of HIV-1 isolates. Thus, the replication capacity of the virus as well as the resistance associated mutations in the reverse transcriptase gene of HIV-1 infected patients were not

similar in function associated with several nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors [82]. Tenfovir is the preferred regimen for adults and adolescents by WHO 2013 guidelines.Genotypically predicted level of drug resistance mutations pattern shown 65% opted for Tenfovir in case of south Indian population [83].

Conclusion

The mutations in the reverse transcriptase gene is the cause of treatment failure by the antiretroviral therapy. The antiretroviral therapy like nucleoside/nucleotide reverse transcriptase inhibitors are used by the patients is directly related with immunological conditions of the patient as well as the mutations in the reverse transcriptase gene of human immuno-deficiency virus-1. The virologic failure is reported when the genotyping analysis has been done. The human immuno-deficiency virus drug resistance mutations was developed due to mutations in the reverse transcriptase gene associated nucleoside/nucleotide reverse transcriptase inhibitors that can be confirmed by genotypic and phenotypic analysis. Only thing is to analyze the mutations in the reverse transcriptase gene during pre-treatment or treatment failure patients those are falling with antiretroviral therapy.

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