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Analysis of mutations in the HIV-1 reverse transcriptase gene among patients receiving RT inhibitor therapy in Papua and West Papua

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ABSTRACT

The prevalence of Human Immunodeficiency Virus type 1 (HIV-1) in Papua is increasing. One of the contributing factors is antiretroviral therapy (ART) treatment failure. Mutations in the reverse transcriptase (RT) gene have been shown to alter the structure of RT, leading to resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). This study aims to identify mutations in the RT coding gene, evaluate their impact on the effectiveness of RT inhibitors, and determine the frequency of HIV resistance to RT inhibitors among people living with HIV (PLWHA) in Papua and West Papua. Analysis of mutations, resistance, and subtypes was conducted using the Stanford HIV drug resistance database (HIVdb). Subtype classification was validated using COMET, NCBI, and GENE2PONE. YASARA and FOLDX were used to construct RT mutant protein structures. Molecular *docking* was carried out using Autodock and PYRX, while visualization was performed using PyMOL and Discovery Studio. In this study, the frequency of HIV resistance among subjects was 28.57% for NRTI-only resistance and 57.14% for combined NRTI and NNRTI resistance. The most common NRTI-associated mutations were S68G, M184V, K65R, V75M, and L74I, while the most common NNRTI-associated mutations were K103N, G190A, P225H, K238T, and Y188L. The presence of these mutations altered binding affinity and molecular interactions between RT inhibitors and RT, thereby reducing of the effectiveness of RT inhibitor drugs in studied population.

Key words: antiretroviral therapy; HIV-1; Papua; RT coding gene.

INTRODUCTION

Papua is one of the endemic regions for Human Immunodeficiency Virus (HIV) in Indonesia, with widespread transmission occurring across nearly all districts. Between January and June 2024, a total of 1,129 new HIV cases were reported in Papua. According to the Ministry of Health (2024), surveillance data on HIV, AIDS, and PIMS in the first semester of 2024 indicate that, of the 598,271 cumulative HIV cases identified in Indonesia from 1987 to June 2024, Papua ranks as the fifth-highest province, with 47,225 reported cases. Antiretroviral Therapy (ART) remains the most effective treatment for managing HIV infection, as it suppresses viral replication and slows disease progression. Reducing viral activity is expected to prolong the life expectancy of people living with HIV (PLWHA) (Tseng *et al*., 2003). Furthermore, ART has significantly improved both the life expectancy and quality of life of PLWHA (Green, 2016). However, the effectiveness of antiretroviral therapy (ART) can be compromised by several

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factors, one of which is drug resistance arising from genetic mutations in the HIV genome (Skowron & Ogden, 2006).

One of the HIV genes that is highly prone to mutation is the polymerase (pol) gene, which encodes the reverse transcriptase (RT) enzyme. RT plays a central role in the HIV replication cycle, particularly in the reverse transcription of viral ribonucleic acid (RNA) into proviral deoxyribonucleic acid (DNA) (Nastri *et al*., 2023). Two major classes of antiretroviral drugs target RT: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). According to the Ministry of Health (2014), combinations of NRTIs and NNRTIs constitute the standard first-line ART regimen. NRTIs act as nucleoside analogs that competitively inhibit RT, thereby terminating DNA synthesis, whereas NNRTIs bind to an allosteric site on RT, inducing conformational changes that impair enzymatic activity (Skowron & Ogden, 2006).

Mutations in the *pool* gene can alter the structural configuration of the RT protein. Thus structural changes may affect protein flexibility, stability, molecular interactions, and binding capacity (Miceli *et al*., 2013). The emergence of such mutations can reduce stabilizing interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces (Sarafianos *et al*., 2009), ultimately weakening the binding affinity between RT inhibitors (RTIs) and RT (Qiu *et al*., 2021). This reduction in binding affinity decreases the effectiveness of RTIs in inhibiting viral replication. Therefore, understanding the relationship between *pol* gene mutations and RT-mediated resistance mechanisms is essential for evaluating the effectiveness of RTI-based therapy.

This study aims to identify mutations in patients receiving RTI-based ART and to analyze their potential impact on drug effectiveness. If resistance to RTIs is detected at the RT level, patients may require timely transition to a second-line ART regimen to maintain therapeutic efficacy. Thus, this research contributes to improving ART outcomes and strengthening HIV control strategies.

MATERIALS AND METHODS

Time and Location

This study was conducted from August 2024 to June 2025. The research utilized DNA sequence data obtained from HIV resistance studies carried out in Papua in 2017 and in West Papua in 2019. All data analyses were performed at the Faculty of Biology, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia.

Materials and Tools

Materials. The materials used in this study included HIV viral sequences, respondent characteristics, the HIV reverse transcriptase (RT) protein structure, and ligand structures of RTI-class drugs. HIV viral sequences and respondent characteristics from 14 PLWHA collected in 2017 and 2019 were obtained from the Papua Public Health Laboratory. Antiretroviral drug protein data were retrieved from the NCBI database. The wild-type RT protein structures were obtained from the RCSB Protein Data Bank with IDs 1RTD (Singh *et al*., 2024) and 1REV (Seal *et al*., 2011). Ligand structures for lamivudine (3TC), zidovudine (AZT), nevirapine (NVP), efavirenz (EFV), and tenofovir (TDF) were retrieved from PubChem with IDs 60825, 35370, 4463, 64139, and 6398764, respectively (Yousefi, 2024).

Tools. The tools used in this study consisted of various software platforms, including COMET (Struck *et al*., 2014), the National Center for Biotechnology Information (NCBI), GENE2PHENE, the Stanford HIVdb Drug Resistance Database, the Calibrated Population Resistance (CPR) tool (Gifford *et al*., 2009), Yet Another Scientific Artificial Reality Application (YASARA) (Ozvoldik *et al*., 2023), FOLDX 5 (Delgado *et al*., 2019), AutoDock Tools (Morris *et al*., 2009), PyRx (Dallakyan & Olson, 2015), PyMOL, and Discovery Studio Visualizer (Baroroh *et al*., 2023).

Sample collection

The samples consisted of 14 HIV viral sequences obtained from patients infected with HIV who were receiving Antiretroviral Therapy (ART) in 2017 and 2019 at the Papua Public Health

Laboratory. Supporting data included respondents' medical records, comprising age, viral load, CD4+ T-cell counts before and after ART initiation, type of ART regimen administered, duration of ART, and patient adherence to ART medication.

Sample processing

All 14 HIV viral sequences were analyzed to determine their subtypes using the online tools COMET, NCBI, and GENE2PHENE (Hasanshahi *et al*., 2023). Drug-resistance mutations associated with ART were identified using the Stanford HIVdb Drug Resistance Database (Gifford *et al*., 2009). The estimated population frequency of sequences carrying resistance-associated mutations was assessed using the Calibrated Population Resistance (CPR) tool.

Construction of mutant RT sequences

The three-dimensional structure of the wild-type RT protein was prepared using Discovery Studio by removing the p55 subunit and the native ligand, leaving only the p66 subunit for further analysis. Following preparation, the wild-type RT protein was mutated according to the mutation profiles identified through the Stanford HIVdb analysis. Mutant construction for each subject was performed using the FOLDX 5 software integrated as a plugin within YASARA. A total of 14 mutant RT proteins were generated from the 1RTD structure and 14 mutant RT proteins from the 1REV structure.

Molecular docking

Molecular docking analysis was performed to determine the binding affinity between HIV RT proteins (as receptors) and antiretroviral (ARV) drugs (as ligands) using AutoDock Tools, PyRx, PyMOL, and Discovery Studio Visualizer (Tarasova *et al*., 2018). The wild-type RT structures (1REV and 1RTD) were prepared in Discovery Studio by removing the p55 subunit and the native ligand. The test RT proteins consisted of mutant structures generated using YASARA and FOLDX.

All wild-type and mutant RT proteins were prepared in AutoDock Tools by removing water molecules, adding hydrogen atoms, assigning Gasteiger charges, and adding AD4 atom types. Molecular docking was conducted using PyRx, pairing each RT protein with its respective ligand. The grid box center coordinates were determined based on the position of the native ligand in the wild-type RT structure, identified using Discovery Studio. Docked ligand conformations were merged with the RT protein structures using PyMOL. Interaction visualization between RT and ligands was performed in Discovery Studio. RMSD values were calculated using YASARA. Redocking was carried out on the wild-type RT structures using their native ligands: TB9 for 1REV and TTP for 1RTD.

Data analysis

Patient clinical data, major HIV mutations, and HIV drug-resistance profiles were analyzed descriptively by tabulating all point mutations identified in each sample.

RESULTS AND DISCUSSION

Characteristics of study subjects

The samples analyzed in this study consisted of DNA sequences obtained from PLWHA who had received counseling and clinical care at Voluntary Counseling and Testing (VCT) centers in Papua and West Papua. A total of 14 samples that had undergone nucleotide sequence analysis were included for further analysis.

The study subjects were HIV-1-infected patients who had been receiving antiretroviral therapy (ART) for 6–48 months. Respondents originated from several regions in Papua, including Nabire City, Jayapura Regency/City, Jayawijaya, Sorong City, and Fakfak City. Sample collection was conducted in 2017 and 2019,

Table 1. Data of respondents infected with HIV and receiving ART (n = 14).

Characteristic	n (%)
Sampling year	
2017	11 (78.6)
2019	3 (21.4)
Location	
Kota Nabire	3 (21.4)
Jayapura Regency/City	3 (21.4)
Jayawijaya	4 (28.6)
Kota Sorong	2 (14.3)
Kota Fakfak	1 (7.1)
Respondent age (years)	
< 25	2 (14.3)
≥ 25	12 (85.7)
Duration of therapy (years)	
< 1	2 (14.3)
1-4	4 (28.6)
> 4	8 (57.1)
Type of therapy	
3TC + ZDV + NVP	2 (14.3)
3TC + EFV + TDF	12 (85.7)
Viral load (copies/mL)	
< 100.000	2 (14.3)
100.000-1.000.000	7 (50.0)
> 1.000.000	5 (35.7)
CD4+ before therapy (cells/µL)	
≤ 200	2 (14.3)
> 200	9 (64.3)
not available	3 (21.4)

resulting in 14 blood samples from which HIV-1 reverse transcriptase sequences were isolated. Patients received combination ART consisting of three antiretroviral drugs. Two patients were treated with lamivudine (3TC) + zidovudine (AZT) + nevirapine (NVP), while twelve patients received lamivudine (3TC) + efavirenz (EFV) + tenofovir (TDF). Overall, most respondents were from Jayawijaya (28.6%), sampled in 2017 (78.6%), aged over 25 years (85.7%), had been on ART for more than four years (57.1%), and predominantly received the 3TC + EFV + TDF regimen (85.7%) (Table 1).

HIV characteristics

In this study, the majority of subjects were infected with HIV-1 subtype CRF01_AE (78.6%), while only 7.1% were infected with subtype CRF02_AG (Figure 1). Specifically, 11 patients carried the CRF01_AE subtype, 1 patient carried the CRF02_AG subtype, and 2 patients were infected with subtype B.

Drug-resistance mutations identified

This study analyzed mutations associated with resistance to NRTI and NNRTI classes of antiretroviral drugs. The NRTI-associated resistance mutations detected in patients included S68G, M184V, K65R, V75M, L74I, M41L, E44D, A62V, T215Y, T69D, K70R, K219Q, K65KR, M184MI, and V75VM. Among these, the most frequent NRTI mutations were S68G and M184V, each occurring at 50.0%. The least frequent NRTI mutations, each appearing in 7.14% of samples, were M41L, E44D, A62V, T215Y, T69D, K70R, K219Q, and V75VM (Figure 2).

NNRTI-associated resistance mutations identified in patients included K103N, G190A, P225H, K238T, Y188L, A98G, E138A, V106M, L100I, K101H, V106I, and Y181C. The most common NNRTI mutation was K103N, with a frequency of 50.0%. The least frequent NNRTI mutations, each occurring in 7.14% of samples, were A98G, E138A, V106M, L100I, K101H, V106I, and Y181C (Figure 2).

Patterns of drug resistance among study subjects

In this study, the majority of patients exhibited resistance to both NRTI and NNRTI drug classes,

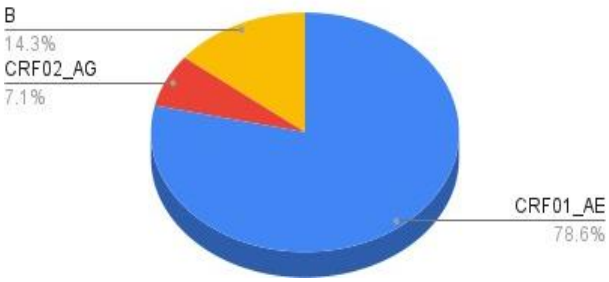

Figure 1. HIV-1 subtypes identified in all study subjects, consisting of three subtypes: CRF01_AE, CRF02_AG, and subtype B.

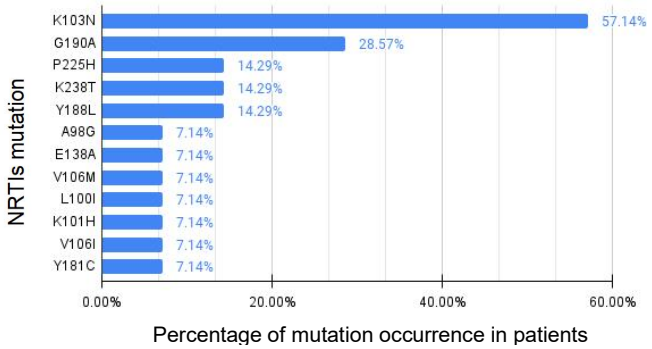

Figure 2. Percentage occurrence of NNRTI mutations.

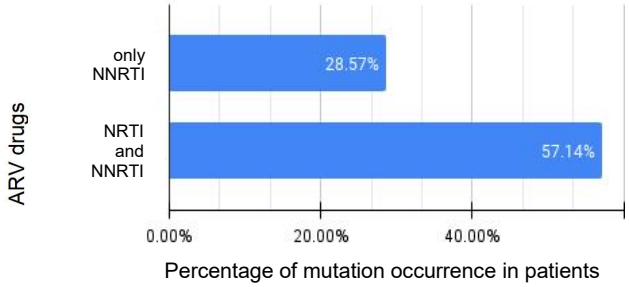

Figure 3. Frequency of drug resistance to ARV drugs.

with a frequency of 57.0%. Patients showing resistance exclusively to NNRTIs accounted for 28.57%, while no patients were found to have resistance to NRTIs alone (Figure 3). Among NRTI drugs, zidovudine (AZT) remained the most susceptible, showing the lowest level of resistance. In contrast, FTC and 3TC displayed the highest levels of resistance (Figure 4). This similarity is expected as FTC and 3TC share highly similar chemical structures, resulting in comparable resistance profiles. For NNRTIs, doravirine (DOR) demonstrated the highest susceptibility, whereas nevirapine (NVP) showed the highest resistance (Figure 5). This pattern is consistent with the pharmacological characteristics of these drugs: DOR is a newer NNRTI with a higher genetic barrier to resistance, while NVP and EFV are first-generation NNRTIs known to have lower genetic barriers. In contrast, etravirine (ETR) and rilpivirine (RPV) are second-generation NNRTIs, which generally possess higher genetic barriers and therefore better resistance profiles compared to first-generation NNRTIs.

Molecular docking analysis of ARVs with RT

In this study, molecular docking was employed as a computational approach to analyze the interactions between ligand molecules (ARV drugs) and receptor proteins (HIV RT). Molecular docking enables the evaluation of how mutations in the RT protein influence ligand binding (Annan *et al*., 2024; Seal *et al*., 2011). The docking analysis aimed to binding affinity (docking scores), and molecular interactions between wild-type RT and ARV drugs, as well as between mutant RT proteins and the same drugs. The ARVs selected for docking corresponded to the treatment regimens used by the study subjects: lamivudine (3TC), AZT, NVP, EFV, and TDF. Based on the docking results, the binding affinities of wild-type RT with 3TC, AZT, NVP, TDF, and EFV were –5.0 kcal/mol, –5.4 kcal/mol, –9.2 kcal/mol, –5.6 kcal/mol, and –10.9 kcal/mol, respectively (Table 2). Several mutant RT proteins exhibited docking scores similar to the wild type, while others showed increased or decreased binding affinity relative to the wild-type values.

Binding between HIV RT and RT inhibitors involves several types of molecular interactions. The key interactions evaluated in this study included hydrogen bonds, hydrophobic interactions, and electrostatic interactions, as these are fundamental to the inhibition mechanism (Almerico *et al*., 2008; Vanangamudi *et al*., 2023). Each interaction occurs at specific amino acid

Table 2. Docking scores between reverse transcriptase and antiretroviral drugs.

Reverse transcriptase	*Binding affinity* (kcal/mol)		
	3TC	ZDV	NVP
	NRTI	NRTI	NNRTI
Control	-5	-5.4	-9.2
Subject 2	-5	-5.5	-9.6
Subject 11	-5	-5.4	-9.2

Reverse transcriptase	*Binding affinity* (kcal/mol)		
	3TC	TDF	EFV
	NRTI	NRTI	NNRTI
Control	-5	-5.6	-10.9
Subject 1	-5	-5.6	-10.9
Subject 3	-5	-5.9	-10.1
Subject 4	-5	-5.8	-9.4
Subject 5	-4.7	-4.9	-10.5
Subject 6	-5	-5.8	-10.2
Subject 7	-4.6	-5.4	-8.8
Subject 8	-5	-5.6	-10.9
Subject 9	-5.3	-5.8	-8.9
Subject 10	-5.4	-6.2	-9.4
Subject 12	-5.4	-5.7	-10.7
Subject 13	-4.8	-5.3	-9.9
Subject 14	-5.8	-5.9	-9.4

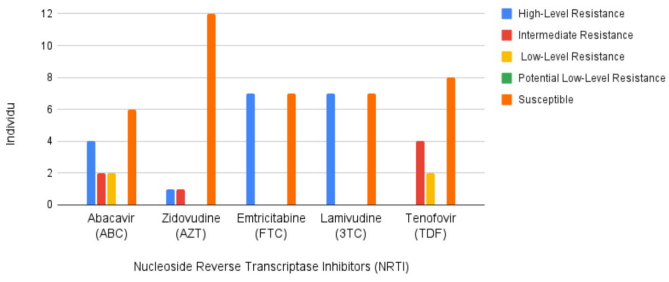

Figure 4. Prediction of resistance to NRTI drugs.

residues, and the present analysis focused on identifying these residues and the interaction types formed during docking.

Molecular docking between wild-type RT and AZT revealed hydrogen bonds at Lys65 and Asp113, hydrophobic interactions at Ala114, Phe116, and Met184, and electrostatic interactions at Arg72 and Asp185 (Figure 6). Docking of wild-type RT with 3TC showed hydrogen bonds at Tyr115 and Gln151, along with hydrophobic interactions at Ala114 (Figure 7). Docking of wild-type RT with TDF demonstrated hydrogen bonds at Lys65, Arg72, Asp113, Als114, and Asp185, as well as hydrophobic interactions at Tyr183 and Met230 (Figure 8). For EFV, docking revealed a hydrogen bond at Gly190, a halogen interaction at Tyr188, and hydrophobic interactions at Leu100, Val106, Val179, Tyr181, Phe227, Trp229, and Leu234 (Figure 9). Docking with NVP showed hydrophobic interactions at Pro95, Leu100, Val106, Val179, Tyr181, Phe227, Trp229, and Leu234 (Figure 10).

Analysis of the impact of mutations on RTI resistance

In molecular docking analysis, it is essential to examine the residues involved in binding between RT and RTIs. Alterations in these interacting residues, such as the loss of hydrogen bonds with key residues or the formation of new interactions with non-critical residues, may indicate changes in the position and orientation of the RTIs within the binding pocket. These structural changes can reduce binding stability, and consequently, diminish inhibitory activity.

Docking results for Subject 1 showed that 3TC, TDF, and EFV maintained interactions with the appropriate key residues and exhibited strong binding affinities, suggesting that these drugs remain effective. In Subject 2, AZT and NVP indicated with differet residues compared to the

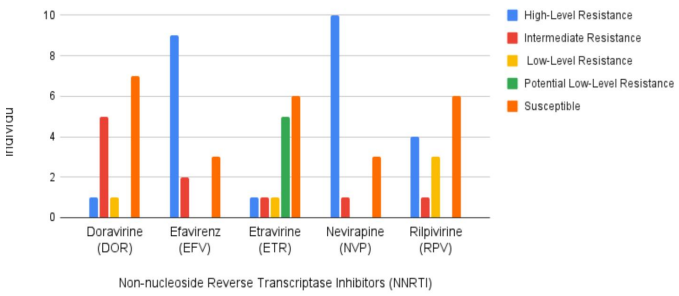

Figure 5. Prediction of resistance to NNRTI drugs.

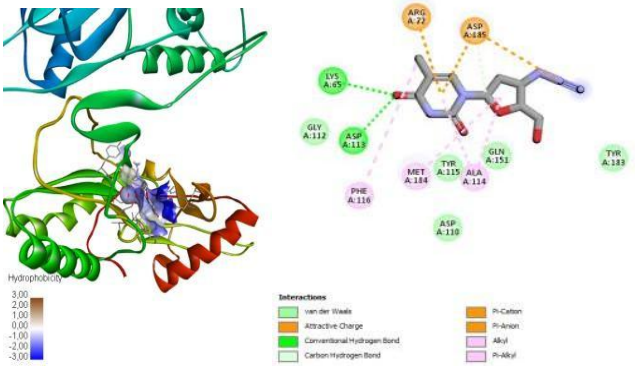

Figure 6. Interaction of wild-type RT protein with the AZT ligand.

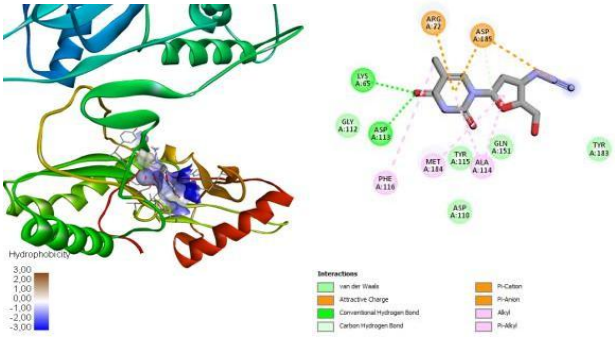

Figure 7. Interaksi protein RT *wildtype* dengan ligan ZDV.

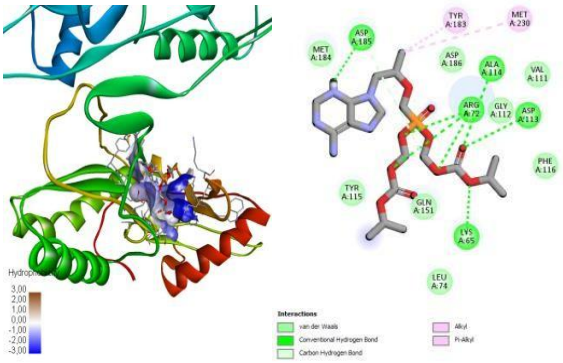

Figure 8. Interaksi protein RT *wildtype* dengan ligan TDF.

wild-type RT, accompanied by loss of hydrogen bonds and reduced binding affinity, indicating decreased drug effectiveness. Although 3TC exhibited a binding affinity comparable to the wild type, its interactions shifted away from key residues and lost critical hydrogen bonds, suggesting reduced effectiveness. For Subject 3, 3TC and TDF maintained interactions with key residues and preserved strong binding affinities, indicating retained effectiveness. In contrast, EFV interacted with alternative residues and showed reduced binding affinity, suggesting decreased effectiveness in this subject.

Docking results for Subject 4-9 revealed heterogenous patterns of RT inhibitor (RTI) interactions and binding affinities. In Subject 4, 3TC and TDF maintained interactions with key residues and exhibited strong binding affinities, indicating preserved effectiveness, whereas EFV showed loss of hydrogen lost hydrogen bonds and hydrophobic interactions, accompanied by a reduced binding affinity, suggesting decreased effectiveness. In Subject 5, all three drugs (3TC, TDF, and EFV) exhibited altered molecular interactions and reduced binding affinities compared to the wild type, indicating decreased effectiveness. Similarly, Subject 7 showed reduced binding affinities and altered interactions for all three drugs, suggesting diminished therapeutic efficacy. In contrast, Subject 6 and 8 retained, retained favorable binding profiles for most drugs. In Subject 6, 3TC and TDF maintained strong interactions with key residues, while EFV displayed reduced binding affinity and altered interactions. In Subject 8, all three drugs maintained interactions with key residues and exhibited strong binding affinities, indicating preserved effectiveness. For Subject 9, 3TC retained strong binding affinity and interactions with the key residues, suggesting continued effectiveness, whereas TDF and EFV showed altered molecular interactions and reduced binding affinities, indicating decreased effectiveness.

Docking results for Subject 10-14 further demonstrated variability in RTI effectiveness. In Subject 10 and 14, all three drugs (3TC, TDF, and EFV) exhibited altered molecular interactions and reduced binding affinities, indicating diminished effectiveness. In contrast, Subject 11 showed preserved interactions with key residues and strong binding affinities for all three drugs,

suggesting retained effectiveness. In Subject 12 and 13, TDF maintained interaction key residues and exhibited strong binding affinity, indicating preserved effectiveness. However, both 3TC and EFV showed altered molecular interactions and reduced binding affinities, indicating decreased effectiveness.

Key residues involved in NRTI and NNRTI binding differ due to their distinct binding sites within the RT protein. NRTIs bind to the polymerase active site, where key residues include Lys65, Arg72, Asp110, Asp113, ala114, Tyr115, Asp185, Asp186, and Gln151. NRTIs typically form hydrogen bonds with residues such as often form hydrogen bonds with residues such as Lys65, Arg72, Leu74, Asp113, and Ala114. Residues Asp110, Asp185, and Asp186 function as catalytic carboxylates that coordinate Mg^{2+} ions which are essential for enzymatic activity. Additionally, Arg72 and Lys65 are involved in binding phosphate groups of deoxynucleoside triphosphates (dNTPs), while Tyr115 acts as a strategic gate that regulates substrate entry into the active site. Residues Asp113, Ala114, Tyr115 Phe116, and Q151 form the pocket that interacts with the 3′-OH group of dNTPs (Barnard *et al*., 2019; Sarafianos *et al*., 2009; K. Singh *et al*., 2010).

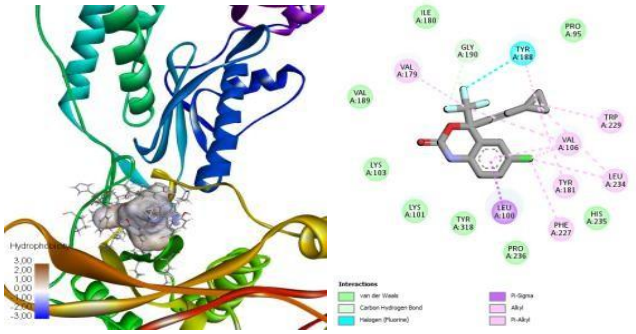

Figure 9. Interaksi protein RT *wildtype* dengan ligan EFV.

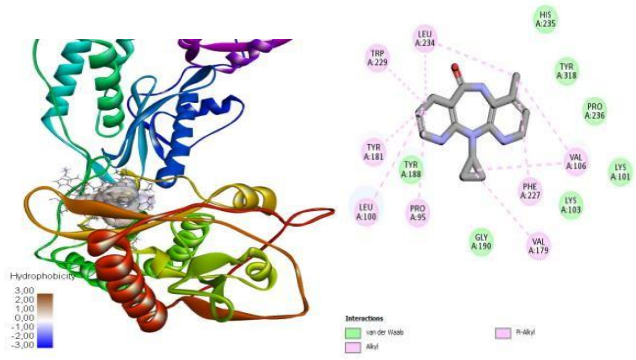

Figure 10. Interaksi protein RT *wildtype* dengan ligan NVP.

Key residues involved in NNRTI binding include Leu100, Lys101, Lys103, Val106, Glu138, Tyr181, Tyr188, Gly190, Leu234, Pro236, Phe227, Trp229, Met230, and Tyr18. Residues Leu100 and Gly190 are located at the central region of the NNRTI binding pocket, while Lys101, Lys103, and Glu138 are positioned at the pocket entrance, where they play a important role in regulating ligand access. Residues Trp229 and Tyr318 contribute to ligand stabilization through aromatic ring interaction NNRTIs through interactions with their aromatic rings. In addition, residues Leu100, Val106, Val179, Gly190, Leu234, and Pro236 are involved in van der Waals interactions, whereas Pro225 participates in electrostatic interactions (Barnard *et al*., 2019; Delviks-Frankenberry *et al*., 2013; Luk *et al*., 2022; Miceli *et al*., 2013; Sarafianos *et al*., 2009; K. Singh *et al*., 2010; Spampinato *et al*., 2024).

Mutations at these key residues can disrpupt critical interaction, including the loss of hydrogen bonds, weakening of hydrophobic contacts, and disruption the introduction of steric hindrance that prevents optimal ligand positioning. Mutations may also obstruct the pocket entrance, limiting drug access to the binding site, and reduce overall binding stability (Vanangamudi *et al*., 2023). In this study, mutations identified within the NRTI binding site included S68G, M184V, K65R, V75M, L74I, M41L, E44D, A62V, T215Y, T69D, K70R, and K219Q. These mutations were associated with altered binding affinities and disrupted molecular interactions between RT and NRTI drugs, contributing to NRTI resistance in the subjects. Mutations identified within the NNRTI binding site included K103N, G190A, P225H, K238T, Y188L, A98G, E138A, V106M, L100I, K101H, V106I, and Y181C. These mutations were associated with changes in binding affinity and molecular interactions between RT and NNRTI

drugs, resulting in NNRTI resistance among the subjects.

CONCLUSION

The mutations identified in the study subjects were classified into NRTI-associated and NNRTI-associated mutations. NRTI-related mutations included S68G, M184V, K65R, V75M, L74I, M41L, E44D, A62V, T215Y, T69D, K70R, and K219Q, while NNRTI-related mutations included K103N, G190A, P225H, K238T, Y188L, A98G, E138A, V106M, L100I, K101H, V106I, and Y181C. Major mutations were the primary determinants of reduced susceptibility or loss of effectiveness of RTI drug classes. A higher frequency of major mutations corresponded to lower RTI drug efficacy in inhibiting RT activity.

The frequency of HIV drug resistance among subjects showed that 28.57% exhibited resistance to NNRTIs only, while 57.14% exhibited resistance to both NRTIs and NNRTIs. Mutations within the RT-encoding gene altered the structural configuration of the RT protein, leading to changes in binding affinity and molecular interactions between RTIs and RT. These structural and interactional disruptions ultimately reduced RTI effectiveness and resulted in RT resistance to RTI drugs.

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