MINISTRY OF EDUCATION AND TRANING

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ROUTINE HIV VIRAL LOAb DS MONITORING AMONG PATIENTS UNDER FIRST-LINE ANTIRETROVIRAL THERAPY IN THE NORTHERN REGION OF VIETNAM

Major: Medical Microbiology
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SUMMARY OF PhD IN MEDICAL THESIS

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INTRODUCTION

Asia – Pacific is one of fastest growing of HIV/AIDS enpidemic area. In 2018, the VAAC reported the cumulative number of alive HIV cases was 210.450, the alive AIDS cases were 102.448, and the cumulataive mortality among HIV patients was 93.990. As well in 2018, the new diagnosed cases were 10.453, and the number of patients under antiretroviral treatment was 135.055 (65% of total people living with HIV).

In the 2000s, in developed countries, viral loads (VL) monitoring has been performed routinely, as well as CD4 cell counts monitoring for treatmet responses assessment. During recent years, World Health Organization (WHO) recommended that clinical and immunological monitoring had many limitations, includes delayed the diagnosis of treatment failure, led to increase the risk of resistance and reduced the chance of success when switching to second-line antiretroviral therapy. To evaluate the outcomes of implementing routine monitoring of HIV viral load in HIV patients under first-line ART at Bach Mai Hospital, we conducted the study: "ROUTINE HIV VIRAL LOADS MONITORING AMONG PATINETS UNDER FIRST-LINE ANTIRETROVIRAL THERAPY IN THE NORTHERN REGION OF VIETNAM" with two following objectives:

1. *Evaluation of performing routine plasma viral load monitoring outcomes and initial feasibility of measuring viral load by DBS method in patients on first-line ARV treatment in Northern Vietnam.*
2. *Describe the virological characteristics of patients with first-line treatment failured at Bach Mai hospital.*

CHAPTER 1: LITERATURES REVIEW

1.1. INTRODUCTION TO HIV/AIDS

1.1.1. Human Immunodeficiency Virus (HIV)

HIV is the pathogen of acquired human immunodeficiency syndrome in human.

1.1.1.1. Virological characteristics of HIV

HIV has 2 types: HIV-1 and HIV-2, they differ in antigen, molecular weight of structural components, duration of infection, infection rate and progression of the disease. HIV-1 is widely present worldwide and is the causative agent of the AIDS epidemic, while HIV-2 is concentrated primarily in West Africa, rarely elsewhere.

1.1.1.2. HIV-1 structure

Under the electron microscope, HIV is a spherical virus (virion) in a sphere shape, 80-100 nm in diameter. The structure consists of 3 layers, from outside to inside.

1.1.2. Replication of the virus

The gp120 molecule of the virus attaches to the CD4 receptor (present on the surface of CD4 T lymphocytes and some other cells) and the coreceptors (CCR5 or CXCR4) of the target cell, thereby leading to a change of the spatial configuration and revealed the location of the action of gp41 molecule involved in the membrane fusion between virus and target cell. Nucleocapside of the virus will be released into the cytoplasm.

1.2. EPIDEMIOLOGY OF HIV/AIDS

1.2.1. Global epidemiology of HIV/AIDS

According to UNAIDS data, as of July 2019, the world had about 37.9 million people (32.7 million-44.0 million) living with HIV. In particular, in

2018, 1.7 million people were newly infected with HIV and 770.000 people died of AIDS, about 36.2 million - 42 million adults and adolescents aged 15 and under living with HIV and about 1.7 million (1.3 million - 2.2 million) children <15 years old.

1.2.2. Epidemiology of HIV/AIDS in Vietnam

Vietnam is one of fastest-growing countries of HIV/AIDS in Asia - Pacific region. The VAAC reported in 2018, the country had 250.000 people living with HIV, 10,453 newly diagnosed cases, higher than in 2017 (9,249 cases) and there were 2.150 death cases due to HIV/AIDS.

1.3. Antiretroviral therapy in Vietnam

In Vietnam, by 2018, there were 436 ARV treatment facilities nationwide, providing treatment for about 138,000 HIV-infected people, which accounted for over 50% of infected people across the country.

1.4. HIV diagnostic tests in Vietnam

1.4.1 The purpose of diagnostic test

- Blood transfusion and tissue and organ transplantation safety
- HIV/AIDS epidemiological surveillance
- Diagnosis of HIV infection
- HIV/AIDS treatment monitoring
- For research purposes

1.4.2 Principles of HIV diagnostic test

- Ensuring confidentiality and voluntariness.
- Pre and post-test counseling.
- Comply with strategies, and testing procedures.
- Ensuring laboratory quality and biosafety.
- Connect with preventive, and care and treatment programs

1.4.3 Testing strategies

- Strategy I (Applies to safe blood transfusion)
- Strategy II (Apply for epidemiological surveillance)
- Strategy III (Apply for HIV diagnosis)

1.4.4. Perform diagnostic tests for HIV infection

In the diagnosis of HIV, the anti-HIV antibody detection test is the standard method for determining HIV status in people over 18 months of age. Virological tests for viral antigen and viral RNA/DNA genetic materials can detect HIV infection during the incubation period, and in case of difficult to diagnose or retest.

1.4.4.1.1. Indirect method: detect the presence of anti-HIV antibodies in blood or secretions to determine the status of HIV infection in adults and children over 18 months of age.

1.4.4.1.2. Direct method: detect the presence of HIV components

- Detect viral nucleic acids: +) HIV-DNA provirus (found in infected cells); +) HIV-RNA (free virus in plasma).
- Detect virus antigen in blood (p24).

1.4.4.1.3. Methods of detecting antigen and antibodies simultaneously, techniques for simultaneous detection of antigens and antibodies: Some biological products using the technology to detect antigen together with virus (p24) and anti-HIV antibody that allow to early diagnose of HIV in the seroconversion phase.

1.5. Monitoring tests for HIV/AIDS treatment

1.5.1 Viral loads testing

There are several technical principles such as RT PCR, Branch DNA (bDNA), NASBA and Real time PCR used in HIV-RNA quantitative assays.

HIV viral load-testing based on the Real time PCR principle, have the advantage of fast techniques, and could be return the results in a few hours. The technology has high sensitivity and specificity rate.

1.5.1.1 The meaning of viral loads testing

The viral load test of HIV-infected people is a virological value that assesses the patient's condition before ARV treatment, many studies showed the characteristics on the determination of the magnitude of HIV viral load, and illustrated that the risk of transmission of HIV is the viral loads in the body of infecvtec people.

1.5.1.2 Measure of viral load by RT-PCR technique

Principles of Real-time PCR technique for detection of HIV infection: Real time PCR is a method that allows amplification and determination of the number of DNA sequences generated in each heat cycle.

1.5.1.3 Dried Blood spot (DBS) method for viral load measurement

The dried blood spot technique uses a dried blood sample instead of plasma. Compared with other methods, this technique has the advantage of being easy to take, safe, easy to store and transport without refrigeration and can be sent via the post system.

1.5.2 HIV drug resistance testing

1.5.2.1 Phenotypic resistance testing

This is an accurate but very complicated and costly method, performed only in a few laboratories around the world. Phenotypic testing is mainly used in research and redefining gene mutations that cause drug resistance. The virus strains are cultured in an ARV-supplemented medium. The result is determined by the ratio of CI 50 or CI 90 (inhibitory concentration of 50 or 90%) of the virus strain to be studied compared to the drug-susceptible wild type.

1.5.2.2 Genotyping resistance testing

Methods for detecting gene mutations associated with resistance to HIV antiretroviral drugs in patients' blood.

Genotypic testing is the common test used to detect HIV drug-resistant.

1.5.2.3 Classification of resistance mutations

The mutation is an alteration of the HIV genome, which leads to a change in amino acids compared to the wild strain, which reduces the virus' sensitivity to ARV drugs. In the results of resistance, mutations are represented by a combination of specific letters and numbers.

1.5.3 T-CD4 lymphocyte counts test

At the beginning, T-CD4 lymphocyte tests were performed using magnetic beads and microscopic counting. Then, the testing based on flow cytometry has become more popular and this method has been considered as a standard method for T-CD4 lymphocytes counting tests so far.

1.6. HIV/AIDS TREATMENT MONITORING

1.6.1 Clinical monitoring

One of the most commonly used assessments to evaluate the disease status and treatment response in HIV / AIDS patients is clinical symptoms and associated opportunistic infections.

1.6.2 CD4 cell counts monitoring

For more than two decades, the CD4 cell count test has been considered as the most basic test for assessing the condition and monitoring the progress of HIV/AIDS patients. CD4 cell count is an important predictor of disease progression and mortality among people living with HIV/AIDS and is a key criterion for assessing the eligibility for antiretroviral drug initiation and prophylaxis therapy, as well as assessment of treatment response.

1.6.3 Viral load monitoring

The viral load is considered as the criteria to determine treatment failure in HIV patients. Although the cost of testing is stilled high, viral load test can help to prevent unnecessary therapy with higher costs (second-line regimen). The latest guidelines from the World Health Organization recommend routine monitoring of viral load, along with CD4 tests and clinical assessments. Studies around the world have also shown that routine viral load testing have cost-effective, and also minimizes treatment burdens in patients with treatment failure.

1.7. HIV DRUG RESISTANCE

HIV drug-resistant is the case of HIV patients under ART but having viral loads value more than 1,000 copies/ml and having HIV drug-resistant mutations. Drug-resistant HIV mutations are the main mutations associated with resistance to one or more of first line ARV drugs.

Drug-resistant HIV is classified into two types: transmitted drug resistance (TDR) and acquired drug resistance (ADR).

CHAPTER 2
MATERIALS AND METHODS

2.1. Subjects

HIV patients under first-line antiretroviral therapy at out-patient clinical, Bach Mai Hospital.

Inclusion criteria:

1. Age $\geq$ 16.
2. Confirmation of HIV postive.
3. Meet the MOH's ARV treatment criteria.
4. Complete the mandatory compliance training
5. Agree to participate and provide informed consent

2.2. Methods

2.2.1. Sample size

All patients registered for treatment at the HIV out-patient clinic, Bach Mai Hospital and eligible for selection criteria were invited to participate in the study. A total of 648 patients agreed to participate in the study with an agreement rate of 90%.

2.2.2. Study design

Prospective cohort study. Patients were followed-up from baseline (before ART initiation) to 36 months of after ART initiation.

a. Plasma viral load monitoring and determine the virological characteristics of patients with first line treatment failure

a. Clinical and laboratory indicators monitoring: Routine clinical examination, laboratory results after 1 month of ART initiation: Complete blood count (CBC), AST, ALT. Each 6 months: CD4 cell counts, CBC, AST, ALT.
b. Plasma viral load: every 6 months after receiving ART for 36 months on 305 subjects. The remaining 343 patients were assessed for viral load at the baseline, 36 months after treatment and the time of suspected for treatment failure accordance with the guidance of the Ministry of Health.
c. Determination of drug resistance genes: only performed in patients with viral load > 1.000 copies/ml.

b. Initially assess the feasibility of measuring viral load by the DBS-PBS method

Randomly select 79 patients in the routine VL group to perform the VL measurement by both plasma and DBS-PBS method at M0, M6, M12, M24 and M36 (M0: before antiretroviral therapy, M6, M12, M24, M36: 6, 12, 24

and 36 months post-treatment). A total of 264 DBS samples were collected in this study.

2.2.2. Study site and duration: The study was conducted at Bach Mai Hospital, Department of Microbiology, Laboratory of Molecular Diagnosis at National Institute of Hygiene and Epidemiology, Oxfort Laboratory of Tropical Diseases Hospital in Ho Chi Minh City from April 2011 to October 2017.

2.2.3. Data analysis

The mortality rate and the occurence rate of resistance genes were calculated by Kaplan - Meier survival analysis. The log-rank test was used to determine the difference between subgroups.

Logistic regression model was used to determine factors related to viral suppression in patients after 3 years of treatment.

2.3. Implementation of testing techniques in the study

2.3.1. Technique for sampling and storage of samples for viral load measurement, DBS-PBS, storage of samples for resistance gene testing, biochemical and CBC tests

a. Prepare and collect sample for measurement of plasma viral load, DBS-PBS

b. Biochemical and CBC tests

Taking blood tests for CBC, AST, ALT, CD4 cell counts.

2.3.2. Biologicals and chemicals, equipment and technical implementation

- Biological kit used in RT-PCR technique to measure plasma HIV-RNA

- Whatman 903r paper, calcium and magnesium free phosphate buffer (PBS) in CAP/CTM S sample tubes

- Sequencing test to identify drug-resistant mutant genes by ABI

3130XL system sequencer

- Biochemical test to explore the liver function

2.3.3. Steps to conduct research content

- Collecting specimens according to the procedure among partcipants at the HIV outpatient clinic, Bach Mai Hospital.

- Implementing tests at base line: CBC, AST, ALT, CD4, viral loads.

- Routine monitoring tests (each 6 months): CBC, AST, ALT, CD4.

- Viral loads: every 6 months after receiving ART for 36 months on 305 subjects. The remaining 343 patients were assessed for viral load at the baseline, 36 months after treatment and the time of suspected for treatment failure.

- Tests to detect HIV drug resistance genes upon treatment failure (based on viral loads results).

- Collecting data using a structural clinical report form.

2.3.4. Laboratory tests

a. Virological tests

- The plasma viral load test, DBS-PBS were performed at the Department of Microbiology of Bach Mai Hospital.

- HIV resistance genes tests: at the Molecular Diagnostic Laboratory of National Institute of Hygiene and Epidemiology - WHO standard for drug resistance testing labs, Oxfort Laboratory of Tropical Diseases Hospital at Ho Chi Minh City.

b. The probe tests of liver function, CBC, immune cells: AST, ALT, CBC, CD4 cell counts tests were performed at Bach Mai Hospital

- Tests to diagnose opportunistic infections: HBV, HCV, CMV, blood culture: performed at Microbiology Department of Bach Mai Hospital.

2.3.5. Technique used in the study

2.3.5.1 Viral loads measurement: detection and quantification of HIV - RNA in plasma, DBS-PBS with COBAS®AmpliPrep / COBAS® Testa®Mana HIV Test, version 2.0 on the COBAS®AmpliPrep system for automatic sample processing and COBAS®TaqMan 48 Analyzer to automatically amplify and detect.

2.3.5.2 Sequencing to identify drug resistance genes

The amplified DNA segments in the sequencing reaction are fluorescent marked dideoxyribonucleotide (ddNTP), so that they emit fluorescent light when passing through the laser illumination system of machine. The color signals will be converted into electrical signals and displayed on the computer screen. Each vertex represents a nucleic acid and each color represents a specific nucleic acid.

2.4. Ethical considerations

Prior to conducting the study, the protocol was approved by BIDMC in Boston, the Scientific and Medical Council of the Bach Mai Hospital, and the Ethics Committee of the National Institute of Hygiene and Epidemiology.

CHAPTER 3
RESULTS

3.1. General characteristics of patients at baseline

The total number of patients participating in the study was 648, men accounted for the majority of the study subjects with 63%. The median age at the baseline was 35.1 ± 8.7 years. Most of subjects were married (72.8%), and 14.2% were single.

The percentage of patients who finished high school is 60.8%, of which 18.3% have completed college or university education. Most people have jobs

with the unemployment rate or currently attending school accounting for only 12.7%.

HIV transmission through sexual contact accounted for most of the study sample with 78.24%. The proportion of people who are transmitted through injecting drugs accounts for only 17.9%. There was 0.46% of patients who transmitted through homosexuality.

Table 3.3. Clinical characteristics at baseline

Clinical characteristics	Frequency (N=648)	Percentage
WHO Clinical Stage		
Stage 1	300	46,3
Stage 2	48	7,4
Stage 3	70	10,8
Stage 4	230	35,5
BMI score		
Underweight	397	61,3
Normal	222	34,3
Overweight	29	4,5

At baseline, the proportion of patients at clinical stage 4 was 35.5% and clinical stage 3 was 10.8%. The average body mass index is 19.7 with the proportion of underweight patients up to 61.3%.

Table 3.4. HIV treatment characteristics

HIV treatment characteristics	Frequency (N=648)	Percentage
Duration from diagnosed of HIV to ART initiation		
<1 month	247	**38,2**
1 - <12 months	236	**36,5**
12 - <36 months	82	12,7
≥ 36 months	82	12,7
ARV regiment at initiation		
AZT/3TC/NVP	186	28,8
AZT/3TC/NVP	81	12,5
TDF/3TC/EFV	357	55,2
Others	23	3,6
Cotrimoxazole treatment	550	84,9

TB treatment	93	14,4

There was 74.7% of patients received ARV treatment within 1 year after diagnosed of HIV, of which 38.2% were treated within 1 month. The most common initial ARV regimen was TDF/3TC/EFV with 55.2%. The proportion of patients receiving Cotrimoxazole prophylaxis was 84.9% and concurrent TB treatment was 14.4%.

Table 3.5. CD4 cell counts and viral load at baseline

CD4 cell counts and viral load	**Frequency (N=648)**	**Percentage**
CD4 cell counts/cm^3		
<100	301	**46,5**
100 - <200	96	**14,8**
200 - <350	186	28,7
350 - <500	52	8,0
≥500	13	2,0
CD4 (Mean ± SD)	162,8 ± 148,6	
Viral load (copies/ml)		
<20	11	1,7
20 - <1000	16	2,5
1000 - <5000	27	4,2
5000 - <10000	22	3,4
10000	572	**88,3**
Viral load (Mean ± SD)	363,951 ± 843,627	

The proportion of patients with HIV advanced stage at baseline was 61.3%, of which 46.5% have a CD4 count below 100 cells/cm^3. The mean of CD4 cell counts was 162.8 ± 148.6 cells/cm^3. The mean of viral load was 363,951 ± 843,627 copies/ml. The proportion of patients with viral load below the detection threshold was only 1.7%, while the proportion with HIV viral load ≥ 10,000 copies/ml was 88.3%.

3.2. Results of routine viral load monitoring

3.2.1. Viral loads and CD4 cell count outcomes

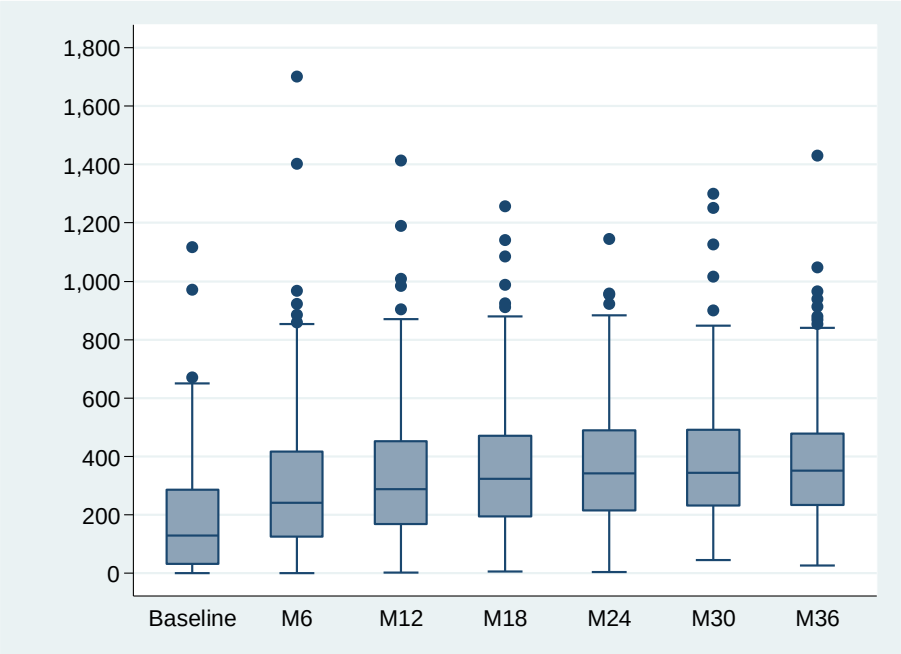

Figure 3.3. CD4 outcomes after 36 months of treatment

At baseline, median CD4 cell count was 130 cells/cm^3 (IQR = 33 - 287). After 6 months, the median CD4 cell count was increased to 242 (IQR = 125 - 417). After 36 months of treatment, the median CD4 cell counts were 352 (IQR = 235 - 478).

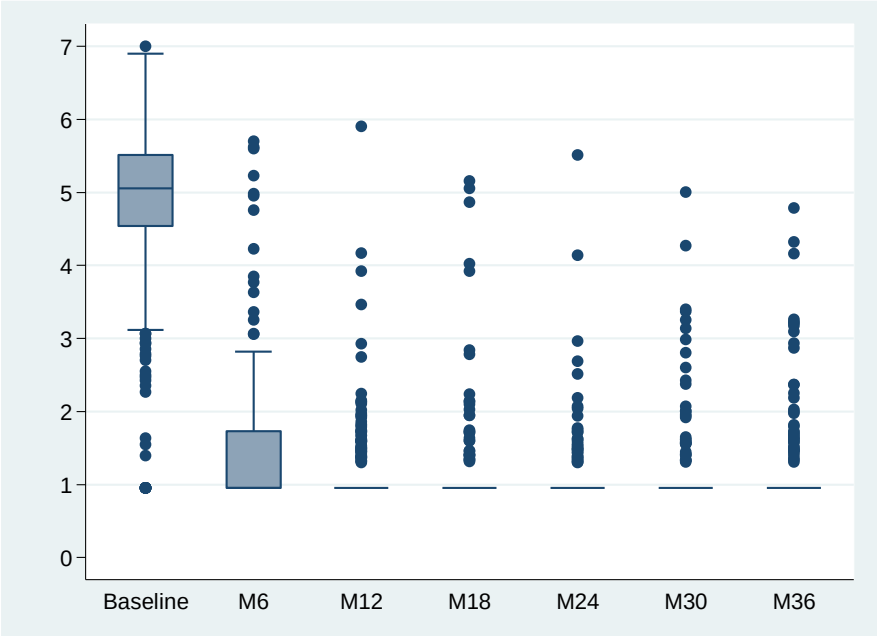

Figure 3.4. Viral loads outcomes after 36 months of treatment

At baseline, the median of log10 viral were 5.05 (IQR = 4.54 - 5.51). After 6 months of treatment, median viral loads were significantly reduced to 0.95 (equivalent to below detection threshold <20 copies/ml) and continued in the following months.

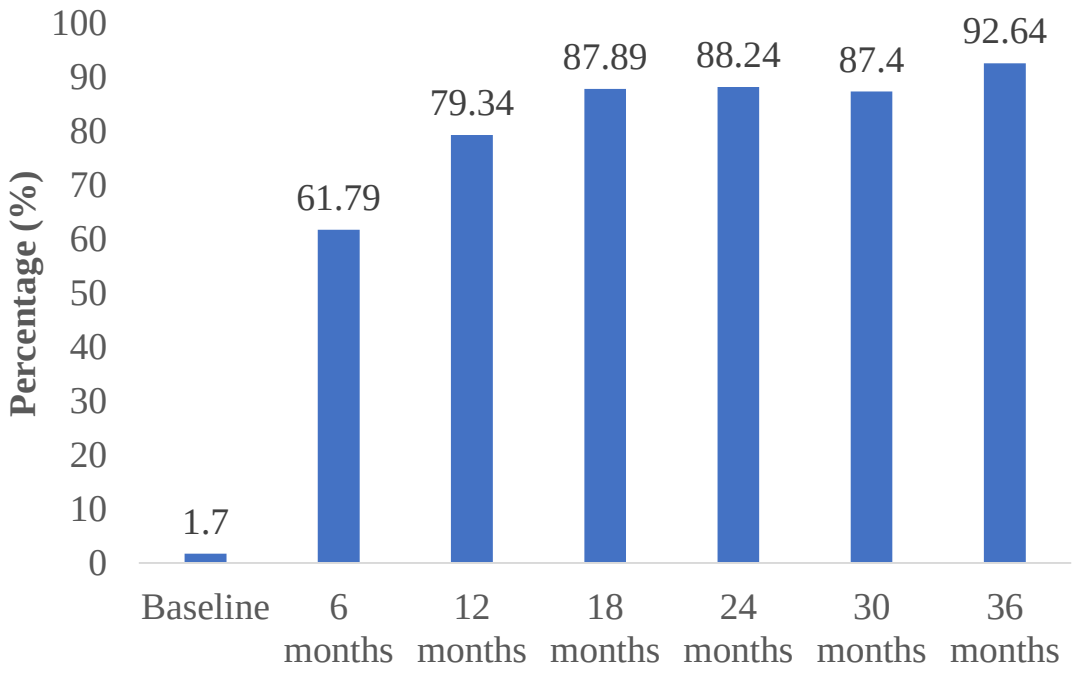

Figure 3.5. Viral load supression rate after 36 months of treatment

The proportion of patients with viral loads below the detection threshold (<20 copie /ml) at the baseline was 1.7%. This rate increased significantly over the first year of ART, with 61.79% after 6 months and 79.34% after 12 months. At 18, 24 and 36 months, the surpression rates were 87.89%, 88.24% and 92.64%, respectively. The Mc-Nemar test showed a statistically significant difference between baseline and subsequent evaluation points.

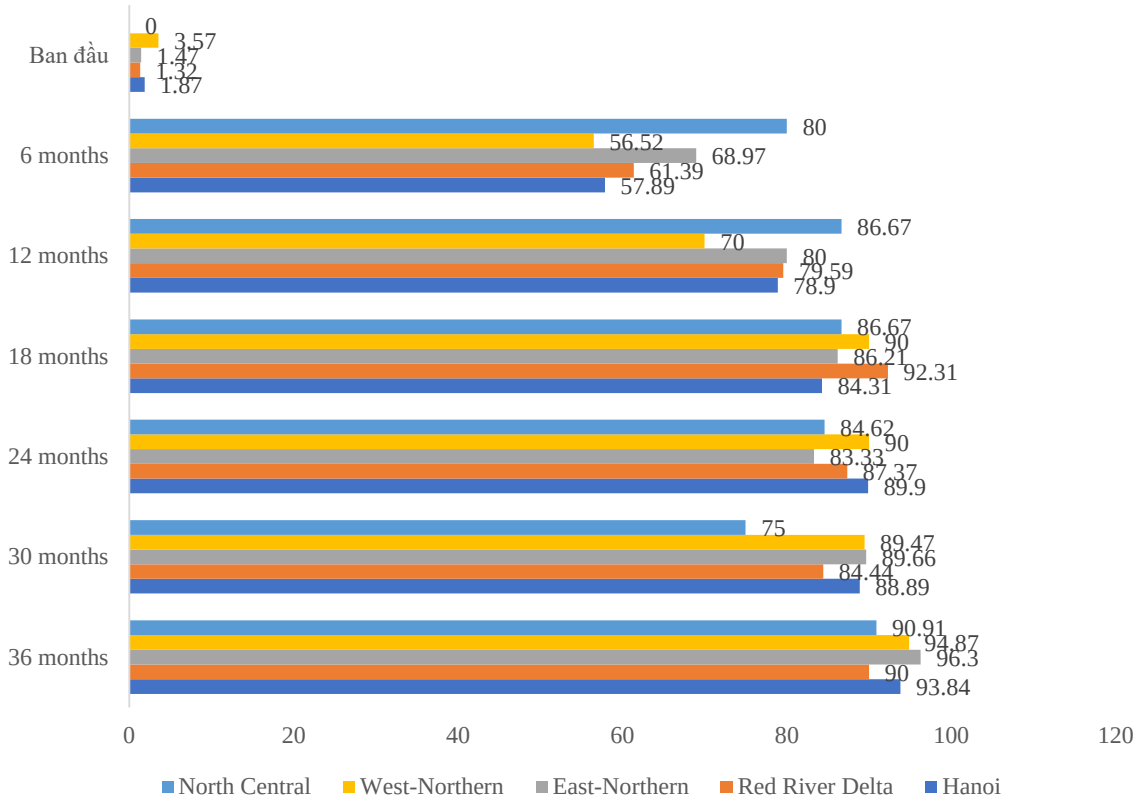

Figure 3.6. Viral surpression rate by region in the North

There was no significant difference in the viral load outcomes by geographic region among patients on first line ART at Bach Mai Hospital. At 6 months, the proportion of patients with VL <20 copies/ml was highest in the North Central region with the rate of 80% and the lowest in the Northwest region with 56.52%, but the difference was not significant. statistical significance (p = 0.45). At the end of the study (36 months after treatment), the proportion of patients with VL surpression in Hanoi was 93.84%, other Red River Delta provinces were 90%, and the Northeast was 96.3%, North West was 94.97% and the North Central provinces was 90.91% (p = 0.43).

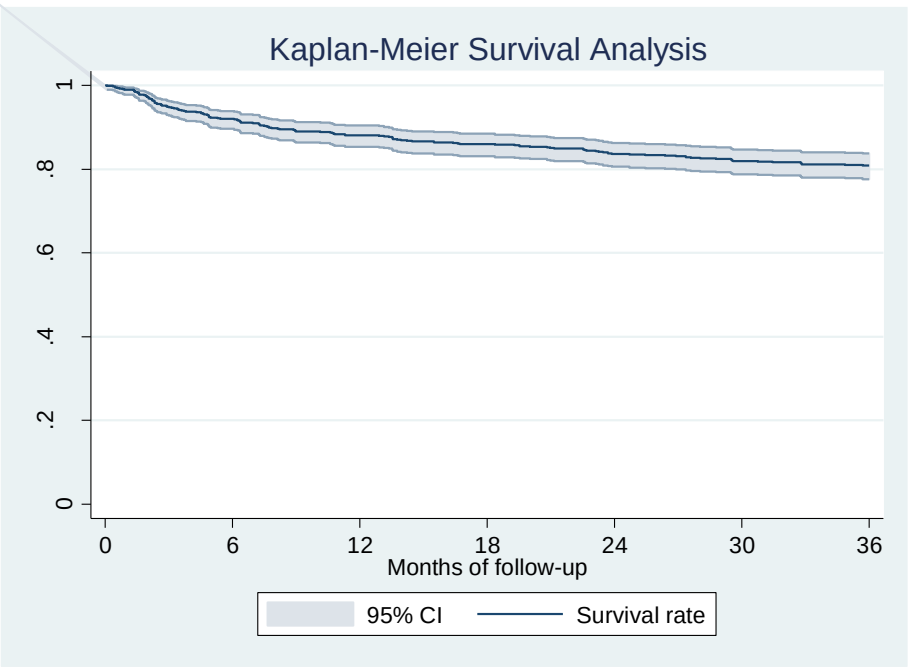

Figure 3.8. Survival outcomes after 36 months of treatment

The survival rate after 6 months of treatment was 92.0%, after 12 months was 88.1%, 18 months was 86.0%, 24 months was 83.6%, 30 months was 81.9% and after 36 months was 79.5%.

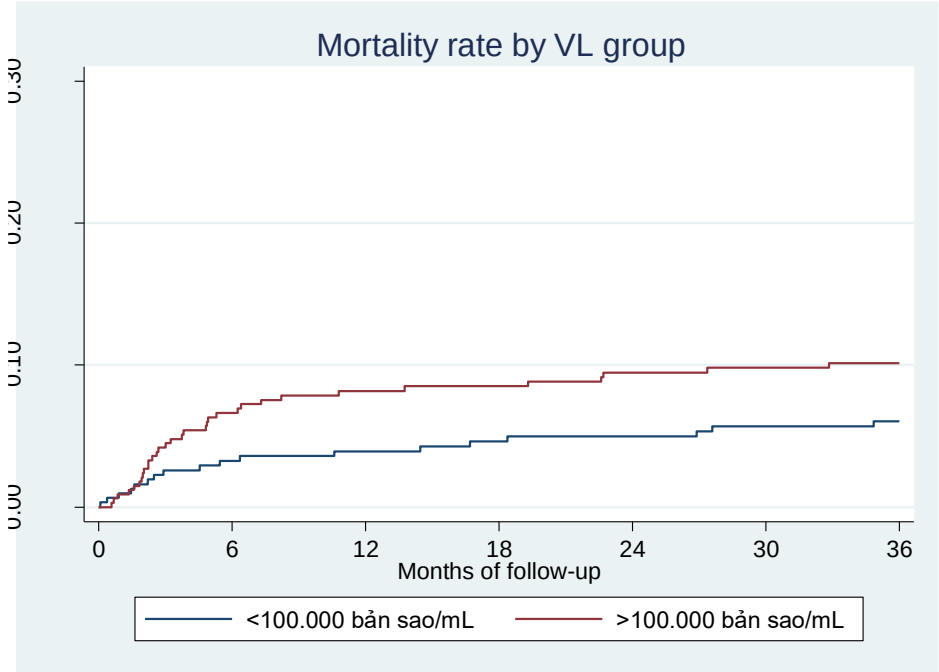

Figure 3.10. Mortality rate by VL group

The overall mortality rate of patients in the study was 2.52/1000 patients-year. Patients with baseline viral load > 100.000 copies/ml have a higher mortality rate than the rest. Log-rank test of test p <0.05.

3.3. Comparison of plasma VL method and DBS-PBS method

Table 3.11. Characteristics of plasma viral load and DBS-PBS at baseline and after ARV treatment

	Before ARV		After ARV		Total (N=264)	
Plasma VL	**n**	**%**	**n**	**%**	**n**	**%**
<20	0	0	145	78,38	145	54,92
20 - <1000	2	2,53	40	21,62	42	15,91
1000 - <5000	1	1,27	0	0	1	0,38
≥5000	76	96,2	0	0	76	28,79
DBS-PBS VL						
<400	4	5,06	178	96,22	182	68,94
400 - <1000	10	12,66	6	3,24	16	6,06
1000 - <5000	24	30,38	1	0,54	25	9,47
≥5000	41	51,9	0	0	41	15,53

The proportion of plasma VL below the detection threshold was 54.92% and <1000 copies/ml was 70.83%. The rate of VL ≥5000 copies/ ml was 28.79%. By DBS-PBS method, the rate of VL <400 copies/ml was 68.94%; from 400 - <1000 copies/ml was 6.06% and ≥5000 copies/ml was 15.53%.

Table 3.12. Sensitivity and specificity of VL DBS method compared to Plasma VL method

DBS-PBS	**Plasma**	
	<1000	≥1000
Threshold of 1000 copies/ml		
<1000	186	12
≥1000	1	65
Sensitivity	99,47%	

Specificity	84,42%	
Threshold of 5000 copies/ml	<5000	≥5000
<5000	188	35
≥5000	0	41
Sensitivity	100%	
Specificity	53,95%	

DBS-PBS has a sensitivity and specificity of 99.47% and 84.42% at the threshold of 1000 copies/ml, however, the specificity was only 53.95% at the threshold of 5000 copies/ml.

3.4. Characteristics of drug resistance and resistance mutations

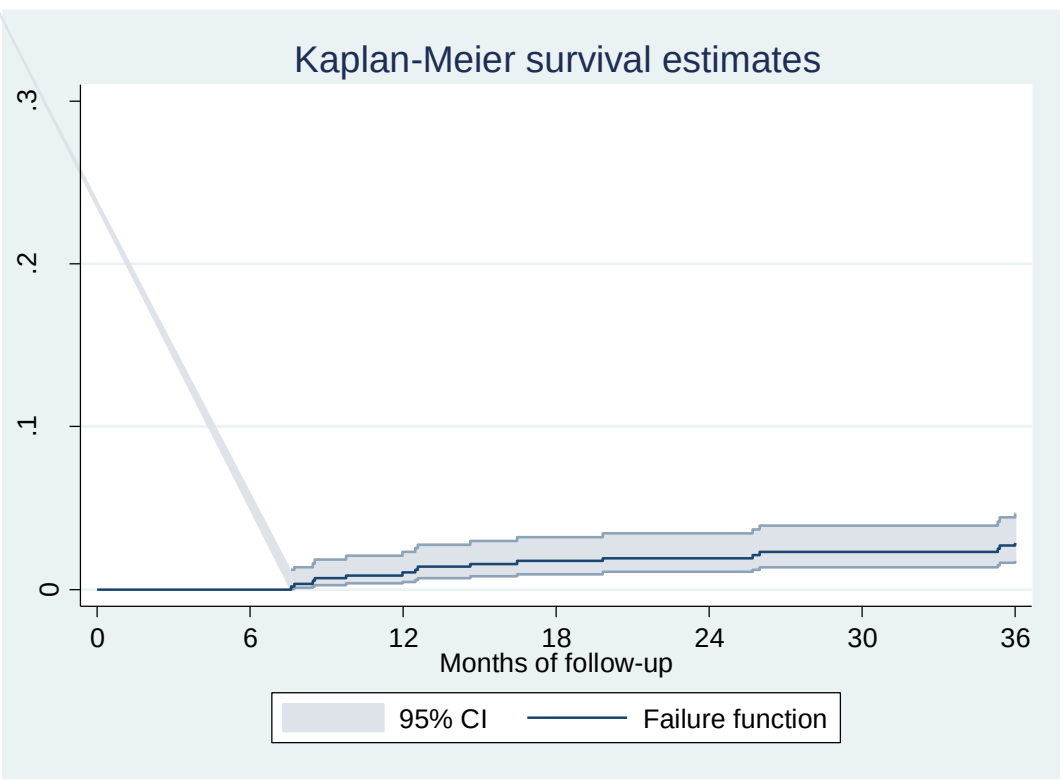

Figure 3.14. Incidence rate of drug resistance after 36 months of treatment

After 36 months of treatment, there were 16 patients carrying the resistance gene with the proportion of 2.47% (6/1648). The incidence rate was 9.76/1000 patient-years. The median time between starting ARV and

confirming the occurence of resistance genes was 1.13 years (IQR = 0.77 - 2.15). Drug resistance in patients first appeared after 6 months of treatment.

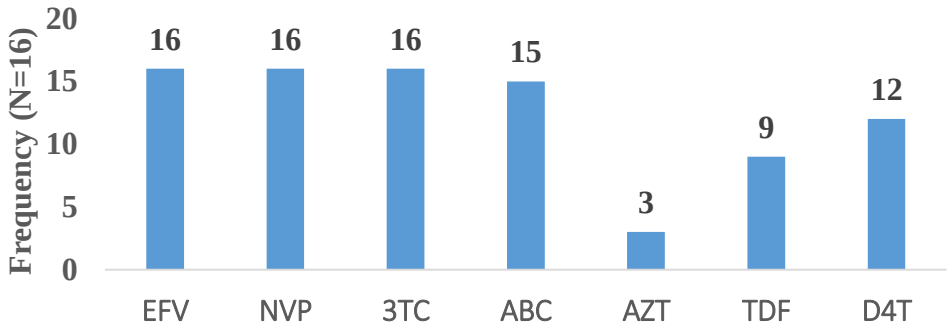

Figure 3.15. The freqency of resistance to common ARV drugs (N=16)

EFV, NVP, 3TC had up to 100% resistance (16/16 cases), followed by ABC with 15/16 cases and d4T 12/16 cases. AZT and TDF have lower resistance rates with only 3/16 for AZT and 9/16 cases for TDF.

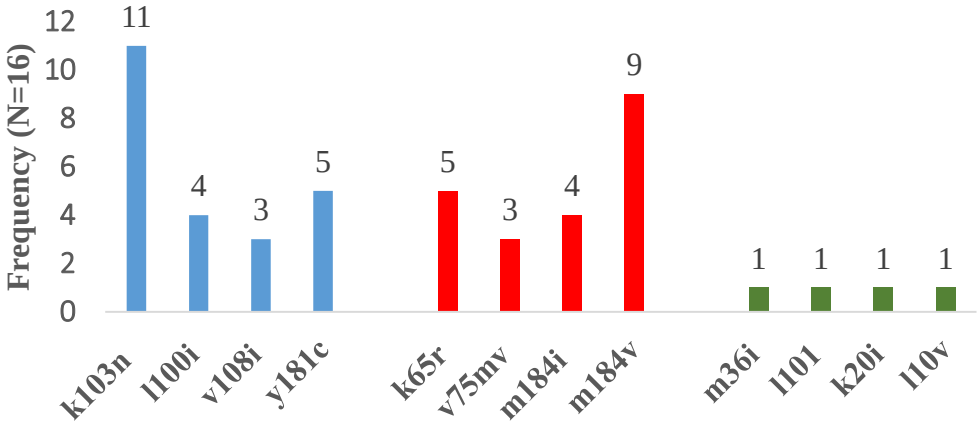

Figure 3.16. Common resistance mutations of each drug class

In NNRTI group, the common mutations were k103n (11/16), y181c (5/16), l100i (4/16) and v108i (3/16). In the NRTI group, m184v was the most common resistance mutation (9/16), followed by k65r (5/16), m184i (4/16) and v75mv (3/16). Four PI-resistant mutation were identified includes m36i, l10v, and a case of having both l101 and k20i muation.

3.5. Factor associated with viral surpression among patients

Table 3.16. Factor associated with viral surpression among patients

Factors	OR	95% CI		p
Gender				
Male	1			
Female	**1,42**	**1,18**	**1,72**	**<0,001**
Age group				
18 - 30	1			
31 - 40	0,93	0,74	1,18	0,57
41 - 50	0,84	0,61	1,15	0,28
>50	0,90	0,63	1,29	0,57
Source of transmission				
Injected drugs	1			
Unsafe sex/Others	0,94	0,71	1,25	0,67
WHO clinical stage				
Stage 3 & 4	1			
Stage 1 & 2	**1,43**	**1,18**	**1,74**	**<0,001**
Smoking				
No	1			
Yes	0,86	0,69	1,07	0,18
Alcohol abuse				
No	1			
Yes	1,00	0,79	1,27	0,99
Hepatitis C				
Negative	1			
Positive	0,93	0,75	1,15	0,49
ARV initiated <4 weeks				
No	1			
Yes	1,05	0,86	1,28	0,62
ARV regimen				
AZT contained/Others	1			
TDF contained	0,97	0,80	1,19	0,80
BMI				
Underweight	1			
Normal	**1,30**	**1,05**	**1,60**	**0,02**
CD4 cell counts (cells/cm^3)				

Factors	OR	95% CI		p
<200	1			
≥200	**3,41**	**2,73**	**4,27**	**<0,001**

Multivariate logistic regression analysis showed that female have higher viral suppression than men (OR = 1.42, p <0.001). Patients had clinical stage 1 and 2 at baseline also had better virological response than patients at stage 3 or 4 (OR = 1.43; p <0.001). Patients with CD4 counts above 200 at baseline were more likely to achieve viral surpression than patients with HIV advance diseases (OR = 3.41; p <0.001).

CHAPTER 4
DISCUSSION

4.1. Demographic characteristics

4.1.1. Age and gender

During the recent years, the age of HIV-infected people on ART in Vietnam and around the world is on the rise, the main reason is that patients have a good response to treatment, therefore the mortality rate and life expectancy have been significantly improved. In our study, the average age of patients was 35.1 ± 8.7 years old, of which, the two highest age groups were the young from 18-30 years old (30, 1%) and the middle-aged age from 31 to 40 years old (49.5%).

Male was accounted for 63%. This result is consistent with the general situation in Vietnam when the HIV/AIDS epidemic between 2010 and 2013 concentrated mainly on injecting drug users, with male gender accounting for the majority.

4.1.2. Marital and employment status

Most of patients currently live with spouses or partners, accounting for 72.8%. This is a great concern because during the last 5 years, the HIV transmission routes in Vietnam has been shifted from injecting drug use to unsafe sex behavior.

There was 12.7% of the patients are currently unemployed, with several are students. Compared to previous studies in Vietnam, the percentage of people in our study is lower, but still a noticeable result.

4.2. Clinical and subclinical characteristics

4.2.1. Clinical characteristics

The proportion of patients at clinical stage 4 was 35.5% and at clinical stage 3 was 10.8%. This result is consistent with many studies in Vietnam from 2013 to 2015 when most of HIV patients came to HIV treatment at a late stage, especially at central level clinics.

The results showed that the majority of patients in our study had severely impaired immune status, and the viral load was very high at the baseline before ARV treatment. Studies in other countries have also shown similar findings that although the average CD4 count before treatment tends to increase, the proportion of patients with low CD4 cell count still accounts for a significant proportion.

4.2.2. Subclinical characteristics

The proportion of patients co-infected with hepatitis C was 36.7%, while the proportion of hepatitis B was lower with 13.1%. This result is consistent with many previous reports in Vietnam when the rate of hepatitis C is particularly high among HIV-infected people. The reason is mainly due to similar characteristics of the transmission route between HIV and HCV among injecting drug users

4.2.3. The role of CD4 cell counts at baseline

We found no correlation between CD4 and viral load at the baseline. This result is consistent with other studies around the world when viral load does not reflect the patient's immunodeficiency status. Assessing the patient's immunodeficiency status plays an important role on initiating treatment interventions at baseline, including prophylaxis treatment of opportunistic infections, especially in late-presentation patients

4.3. Viral load measurement by DBS method

Viral load measurement using DBS method is increasingly popular in the effort of expanding HIV viral load monitoring in Africa and Asia. The advantage of DBS is that it is easier to collect, convenient to store and transport compared to plasma method. Based on reports of a systematic review from 43 studies, WHO recently recommended that the threshold for detection of treatment failure with DBS method to be 1,000 copies/ml. However, the sensitivity and specificity of DBS still varied depending on the background of the VL test. In addtion, the specificity of the DBS test in the case of patients with very lower viral load has been questioned, why DBS can return the viral load results higher than plasma method.

In Vietnam, our study is one of the first studies to evaluate the performance of DBS method compared to the traditional plasma method being applied routinely at HIV clinics. DBS-PBS method results show a sensitivity of 99.47% and a specificity of 84.42% at threshold of 1000 copies/ml, however, the specificity is only 53.95% at threshold of 5000 copies/ml. The results showed that DBS method will have higher practical significance when measuring the viral load after ARV treatment. This finding is similar to some studies that have been done around the world. Ning Tang and his colleagues conducted a study on 497 HIV patients in Ivory Coast,

showing that the sensitivity of the DBS method can reach 93% and the specificity is 95% at the threshold of 1000 copies/ml according to WHO recommendations. In our study, the correlation between DBS assay and plasma method also achieved good results with correlation coefficients of 0.896 and 0.9901 ($p < 0.001$), respectively.

4.4. Viral load monitoring outcomes and associated factors

4.4.1. Viral load monitoring outcomes

Our results showed that patients have a very good viral load outcomes after 36 months of antiretroviral. The median of viral load has been decreased to below the level of detection within the first 6 months of ART. The rate of patients who achieved viral suppression below the detection threshold (<20 copies/ml) after 36 months was up 92.64%. This result is higher than some studies reported in the United States and Europe when the rate of virus suppression is only 58 - 85%. In Vietnam, Suresh Rangarajan et al's study of 1255 adult patients on ART for at least 1 year in 4 provinces reported a 93% rate of patients with viral load <1000 copies/ml, which was higher than our result. Another study conducted by Vu Quoc Dat and colleagues on 365 patients in 16 outpatient clinics nationwide reported a 95.1% rate of viral suppression after 36 months. A study by Junko Tanuma and colleagues conducted in 2017 also showed similar results when the rate of patients had viral supression after 12 months was 95.5%. However, the detection threshold in these studies is 1000 copies/ml, which was much higher than the detection threshold of 20 copies/ml in our study.

In the last decade, the expansion of ARV treatment in low-resourced countries required a simpler approach. Due to insufficient laboratory capacity, many programs have reduced follow-up of laboratory monitoring to optimize the treatment expansion. However, with the development of technical

equipment and resources for HIV, viral load has gradually become a golden standard in monitoring and evaluating the response to antiretroviral therapy in HIV/AIDS people. The latest guidelines from the World Health Organization recommend routine monitoring of viral load, along with CD4 tests and clinical assessments.

The high proportion (> 90%) of patients in the study who achieved viral suppression after 36 months of ARV treatment showed the effectiveness of the treatment program in Bach Mai Hospital and the prospect of meeting 90 - 90 – 90 goal set by the Ministry of Health.

4.4.2. Factor associated with viral surpression

Logistic regression analysis showed that poor clinical condition and severe immunodeficiency before treatment are associated with poorer virological response. Specifically, patients with T-CD4 lymphocyte counts <200 cells/cm^3 and at clinical stage 3 or 4 were more likely of not having viral suppression than others. This is an consistant reflection of conclusion from studies around the world where the viral load > 100,000 copies/ml is strongly associated with decreased CD4 cell count. and progress to the AIDS. Rangarajan and Tanuma also reported similar results when patients with advanced HIV had significantly lower treatment responses. This is the result of the late diagnosed and register to treatment among HIV patients. Many studies in Vietnam have shown that, although ARV treatment standards have been expanding rapidly recently, patients with late treatment stilled account for a high proportion. This result can be explained by a combination of social factors, including fear of stigma and discrimination and also the clinical status. This situation needs to be improved quickly, especially in the context that Vietnam is deploying ARV to district level with lower capacity compared to central level facilities. Other factors related to the viral load surpression

includes gender and nutritional status. Male were more likely to have a lower virological response than women, and underweight patients (BMI <18.5) also have lower viral load surpression than patients with normal BMI. Adherence is an important factor of ARV treatment response. However, in our study, there was no difference in suppressing viral load between treatment compliance levels among patients.

4.5. Drug resistance gene

4.5.1. Resistance rate

In our study, there were 16 cases of treatment failured and switched to second-line regimens, 100% of patients with treatment failured had resistance to at least one drug. Among 16 failured patients, the resistance rate was very high in NNRTI group, of which, EFV and NVP had 100% resistance rate. In the NRTI group, 3TC and ABC drugs have high resistance rates. Most PI drugs were not reported to be resistant, with only three cases carrying the resistance gene.

4.5.2. Resistance mutation gene

The M184V mutation was encountered at a very high rate, accounting for 56.25% (9/16) among patients with NRTI-resistant, possibly due to all the first line regimens contained Lamivudine that is a selective drug to this mutation.

The most common NNRTI resistance mutations were Y181C/V (8 patients), K65R (5 patients), and V75MV (3 patients). Theses were all mutations associated with cross resistance in the NNRTI group, may help to explain the high resistance of most NNRTI drugs

CONCLUSION

1. Evaluation of performing routine plasma viral load monitoring outcomes and initial feasibility of measuring viral load by DBS method in patients on first-line ARV treatment in Northern Vietnam

- The proportion of patients had viral supression (<20 copies / mL) at baseline was 1.7%. This percentage increased significantly after ARV initiation. At 6, 12, 18, 24, 30 and 36 months after treatment, the proportion of patients with viral surpression were 61.79%, 79.34%, 87.89%, 88.24%, 87.4% and 92.64%, respectively.
- At the threshold of 1000 copies/ml, the sensitivity and specificity of DBS-PBS method were 99.47% and 84.42%, respectively. However, at 5000 copies/ml, the specificity of DBS-PBS method was only 53.95%. This shows that DBS will have a higher practical significance when measuring viral load during follow-up.

2. Describe the virological characteristics of patients with first-line treatment failured at Bach Mai hospital

- After 36 months of treatment, there were 16 patients carrying the resistance gene with the proportion of 2.47% (6/1648). The incidence rate was 9.76/1000 patient-years. The median time between starting ARV and confirming the occurence of resistance genes was 1.13 years (IQR = 0.77 - 2.15). Drug resistance in patients first appeared after 6 months of treatment.
- The HIV strains carrying the mutated genes in NNRTI group were k103n, y181c, l100i and v108i; in NRTI group, the genes most mutated genes were m184v, k65r, m184i and v75mv; 4 PI resistant genes were m36i, l10v and k20i.

LIST OF PUBLISHED SCIENTIFIC ARTICLES RELATED TO THE THESIS

1. Thai Phuong Truong, Mai Nguyen Thi Tuyet, Minh Vuong Bui, Duy Cuong Do, Tuong Van Vu (2018), "Investigation of the correlation between CD4 cell count and HIV viral load on HIV/AIDS patient before art initiation at Bach Mai hospital, 2012-2015", *Journal of Vietnam Preventive Medicine*, Vol.28 (11), pp. 93-99.
2. Truong Thai Phuong, Le Thi Ngan, Le Trung Dung, Do Duy Cuong, Vu Tuong Van (2018), "Assessment of HIV viral load and relating factors among HIV/AIDS patient treated at Bach Mai hospital, 2012-2015", *Journal of Vietnam Preventive Medicine*, Vol.28 (11), pp. 100-109.