# AN ASSESSMENT OF THE OUTCOMES OF HIV-INFECTED PATIENTS ON SECOND-LINE ANTIRETROVIRAL THERAPY IN HTAR, KLANG

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**Abstract: Introduction:** Human immunodeficiency virus (HIV) drug resistance has been a significant contributor to failure of combined antiretroviral therapy (cART). Once the resistance has occurred, combating it is extremely difficult given the presence of minority variants that may have selective advantage. Patient failing a cART regimen mostly have drug resistant mutations. Optimal and prolonged viral suppression has been shown to significantly reduce the risk of accumulation of resistant variants, hence prolongs duration of effectiveness of the regimen. **Objective**: To assess the virologic, immunologic, clinical and safety outcomes among patients on second-line cART and to describe the patterns of HIVDR mutations among these patients. **Methodology**: The study was a descriptive retrospective review to evaluate the outcomes and the drug resistance pattern of PLHIV among patients on second-line cART at HTAR, Klang, Malaysia. **Results and discussion:** A total of 47 patients were evaluated for outcomes on second line cART. Majority of them (76.6%) was changed to second-line due to treatment failure, 17% due toxicity and 6.4% had psychiatric symptoms. The median (IQR) viral load level decrease at 6 months to 20 (20-109) copies/mL from 20,022 (2208-123845) copies/ml at the time of starting second-line cART; the median (IQR) CD4 cell count at 6 months increased to 291 (220-497) cells/mm$^3$ compared to the time of change 203 ((50-425) cells/mm$^{3.}$ At 6 months there was a significant mean increase of weight by 1.8 kg ± 5 (95% CI: 0.7- 4.9) (P = 0.01). Among patient with treatment failure, 42% (17/36) had drug sensitivity testing: 16.7% (3/17) had single class mutation, 70% (12/17) had double class mutation while 12% (2/17) had triple class mutations. Overall, 88% (15/17) had at least one resistant mutation. **Conclusion**: Assessment of outcomes of patients on second-line cART is necessary to ensure treatment goals are achieved and to safeguard the limited options of antiretrovirals available after second-line therapy.

**Keywords:** Human immunodeficiency virus (HIV), antiretroviral therapy (cART), CD4 cell, HIV drug resistance (HIVDR).

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## INTRODUCTION

Human immunodeficiency virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) is one of the most devastating health related conditions globally. Since it was first recognized in 1981, more than 70 million people have been infected cumulatively and approximately 35 million people have died of HIV/AIDS related illness (WHO, 2016). It is estimated that there were around 37 million people globally living with HIV (PLHIV) by the end of 2017 and there were approximately 1.8 million new infections with nearly 1 million AIDS related deaths during the same period. Asia and pacific region has 5.3 million PLHIV with over 75% of them residing in China, India and Indonesia (UNAIDS, 2018; WHO, 2018). In Malaysia, the number of people cumulatively infected with HIV since 1986 is approximately 115,263 with estimated 87,122 of people living with HIV (PLHIV) by the end of 2017. During the same period, the number of PLHIV who were on combined antiretroviral (cART) were 29018 ( MOH, 2018). The HIV epidemic in Malaysia is concentrated among people with high risk behaviors including injecting drug users (IDU), sex workers, transgender and men who have sex with men (MSM). HIV infection been seen mainly in adults aged between 15-49 with more than 70% new infections reported among 20-39 year old (Zain et al., 2017). The epidemic is mainly among the men as more than 90% of the cases cumulatively are men while females account for less than 10% of the total number (Choy, 2014). In the initial period of the epidemic, IDUs had the highest proportion of total HIV infection with 70-80% of all reported cases from this group. From 2011 there was a shift as the new infections reported were mainly via sexual transmission. (MOH, 2018).

HIV has high production and replication rates which slowly over long periods of time results in diverse strains which results in resistant mutation. Majority of PLHIV will likely require a change in regimen in the course of their treatment because of the eventual development of resistance (Stadeli & Richman, 2013). Optimal and prolonged viral suppression has been shown to significantly reduce the risk of accumulation of HIV resistant variants and thus halt or significantly slow down the development of resistance strains. Once the resistance has occurred, combating it is extremely difficult given the presence of minority variants that may have selective advantage when a cART regimen making the resistant variant the dominant population and may subsequently result in treatment failure (O'Connor et al., 2017). Treatment options for patients failing second-line regimen, treatment options are largely non-existent. The world health organization (WHO) has some guidance in cases second line treatment options but with caution as many countries do not have the capacity to implement the third-line options due to financial limitations (WHO, 2016).

HIV has a significantly high production and replication rate. A person infected with HIV who is not on treatment produces at least $10^{10}$ viruses per day, the virus mutation varies at individual level hence there is a marked variability of disease progression among the patients (Ho et al., 1995; Markowitz et al., 2003). The combination of at least three antiretroviral agents (cART) from three different classes also known as highly active antiretroviral therapy (HAART) has been shown to dramatically suppress viral replication and reduce plasma viral load resulting in reconstitution of the immune system. cART has been shown to substantially improve survival, quality of life, reduces incidence of opportunistic infection and costs making HIV a manageable chronic disease. Viral suppression also reduces the risk of transmission (Grubb et al., 2014). The efficacy of cART regime depends on the activity of the individual drugs in the regimen and the genetic barrier to resistance which is the number of HIV mutations required for the development of resistance for each antiretroviral (Tang & Shafer, 2012b). Optimal pre-cART counselling should be carried out before initiation to ensure patients understanding as this has been shown to result in improved treatment outcome. WHO recommends close monitoring in all patients but more aggressive surveillance in patients who have failed a regimen because of the increased risk of accumulating resistant HIV variants as they have increased risk of regimen failure (Ndembi et al., 2016). Due to the difficulty in combating and predicting the risk of further accumulation given the presence of some resistance in patients using second line cART, there is a need to closely monitor and frequently to assess the status of these patients. The data from this study will therefore provide information to help the policy makers Hospital Tengku Ampuan Rahimah (HTAR), Klang (HTAR)- infectious disease clinic develop evidence-based action plan and may also help forecast the need for treatment options beyond second line.

## METHODOLOGY

**Study Design:** The study was a retrospective study. All patient's data were taken from the infectious disease (ID) clinic, Hospital Tengku Ampuan Rahimah (HTAR), Klang. The purposive sampling was used to select all patients who were on second line Cart. Medical records of patients who had been switched to a second line cART (a protease inhibitor-based regimen) were obtained. All information regarding a patient were documented in individual patient file. Ethical approval was obtained from Medical Research and Ethics Committee (MREC) and the site approval was obtained from HTAR before starting any study related activities with registration no. NMRR-18-3477-45487. The data collected comprise of patients' demographic characteristics (e.g., age, gender), ethnicity, as well as disease relevant information like weight, viral load, CD4 count, ART regimen, viral resistance testing results, compliance information among others.

**Virologic outcomes:** Adequate virologic response was defined as plasma VL <400 copies/mL, VL between 400-1000copies /mL was defined as on-going viremia. Virologic failure for this study was defined as either incomplete initial response to therapy resulting in failure to attain HIV viral load <1000 copies/mL in 4-6 months after starting ART or virologic rebound with persistent HIV viral load > 1000 copies/mL in a patient who was previously suppressed on the same regimen (WHO, 2018). Patients who were recently switched to second line and had no investigations after the switch at the time of the study were censored at the time of at virologic outcome analysis.

**Immunologic outcomes:** Immunologic failure was defined as a drop in CD4 cells to less than baseline pre- second line levels or persistently < 100 cells/mm3 after at least 12 months of second line cART without concomitant or recent infection to cause a temporary reduction in CD4 cell count or the CD4 fell by 30% or more from the peak value achieved while on second line (Patel et al., 2013; WHO, 2018). Any patient who did not achieve any of the above criterion was categorized as having successful immunologic outcome.

**Weight outcomes:** Early weight gain after cART initiation among underweight and normal weight patients is associated with improved survival and reduced risk of clinical failure. It indicates reduced metabolic demand as a result of effective suppression of viral replication and CD4 cell recovery and hence improved clinical outcomes. Generally, uncontrolled HIV is associated with fat loss and poor appetite resulting in weight loss (Grinspoon et al. 2003). There is no consensus of specific definition of weight gained but studies have reported a mean weight gain ranging from 1 - 2.5 at 6 months of change of cART and was associated with mortality benefit (Patel et al., 2013). In this study successful weight gained was defined as weight > 1kg at 6 months of second line cART.

**Safety outcomes:** Viral load is the gold standard for monitoring of patients' outcomes (WHO, 2016). Successful virologic outcomes is defines as VL<400 copies/mL after 6 months of cART while immunologic success is defined as CD4 count >100 cells/mm3 during the same period. Remaining active in care was considered successful treatment outcome. Those transferred out to other facility were not included in the description of retention in care.

Resistance mutation

Resistance mutation in HTAR is classified according to international AIDs society (IAS) guidelines. The results of the

DST contain the mutations identified for each ART class and interpretation that shows the level of resistance is also provided. The interpretation uses the latest Stanford guidelines for HIV genotyping testing (http://hivdb6.stanford.edu). The Stanford HIV drug resistance database is used to determine the level of resistance. The database has a mutation scoring, each mutation is given a score based on the Stanford's internal algorithm. The score determines the level of resistance, the higher the score the greater the resistance. "Susceptible" implies no evidence of reduced ARV susceptibility compared to wild type; low-level resistance indicates that the virus may have reduced in vitro ARV susceptibility or that patients may have a suboptimal virologic response to treatment with the ARV. Intermediate resistance implies that the activity of the drug is reduced but it will likely remain with significant antiviral activity. High level resistance indicates that the predicted level of resistance is similar to those observed in viruses with the highest levels of in vitro drug resistance of that clinical data shows that patients with such mutations have little or no virologic response to the treatment with the ARV (Stanford HIV resistance database). This was used to describe the patterns of the resistant mutations.

**Statistical Analysis**

The data was presented as mean ± standard deviation (SD) for continuous variables and frequency with their respective percentages for categorical variables. Patient characteristics was described using median with inter quartile range (IQR) for skewed continuous data. Wilcoxon Signed Rank Test was used to compare the mean VL and CD4 cell count and weight before and after cART change to second line cART. Mann- Whitney test was used to compare CD4 count in different age groups. The HIVDR was described using percentages. All statistical analyses will be performed using SPSS version 23

## RESULTS

**Characteristics of The Study Subjects**

A total of 47 HIV-infected patients who received second line cART were included in the retrospective study. The means age of the patients is (41.23±9.015) years (ranging from 18-59 years). Most of the patients were male, Malay and married. The risk factor for HIV infection was mainly heterosexual. The most reason for regimen switch from first line to second line were due to treatment failure which had virologic failure (VL>1000copies/mL). Treatment failure was mainly due to poor adherence, 72% (26/36); crushing of tablet, 2.7% (1/36) while 2.7% (1/36) had both poor adherence and was also using herbal medications. Treatment failure for 25% (9/36) was not documented, of these, 7 claimed to have good adherence to their cART regimen therefore the treatment failure was likely due to the development of resistance as a natural course of HIV. Medications toxicity as a reason for change to second line occurred in 19.1% (9/47). Skin reaction suspected to be due to EFV was the most common reason with severe skin rash occurring in 55.5% (5/9), drug rash with eosinophilia and systemic symptoms (DRESS) syndrome and Erythema multiforme occurring in 11% (1/9) each. Others were hypercholesterolemia in 11% (1/9) due to EFV and transaminitis, 11% (1/9) due to NVP. Among those included in the study, those with a previously diagnosis of tuberculosis while on first line cART was 27.7% (13/47); 2% (1/47) had evidence of co-infection with hepatitis B and 10.6% (5/47) hepatitis C infection.

**Table 1.** Socio demographic characteristics.

| Characteristic | Frequency (%) |
|---|---|
| **Age**, mean (SD) (Range) | 41.23 (9.015) |
| **Weigh**t, mean (SD) | 56.95 (11.67) |
| **Gender** | |
| Male | 29 (61.7%) |
| Female | 17 (36.6%) |
| Other | 1 (2.1%) |
| **Ethnicity** | |
| Malay | 19 (40.4%) |
| Chinese | 15 (31.9%) |
| Indian | 12 (25.5%) |
| Other | 1 (2.1%) |
| **Marital status** | |
| Single | 14 (29.8%) |
| Married | 23 (48.9%) |
| Widowed | 7 (14.9%) |
| Divorced | 3 (6.4%) |
| **Risk factor for HIV infection** | |
| Heterosexual | 31(65.9%) |
| Homosexual | 5 (10.6%) |
| IVDU | 5 (10.6%) |
| Bisexual | 1 (2.1%) |
| Vertical transmission | 1 (2.1%) |
| Unknown | 4 (8.5%) |
| **Reason for change to second line** | |
| Treatment Failure | 36 (76.5%) |
| Toxicity | 8 (17.0%) |
| Psychiatric symptoms | 2 (4.3%) |
| Psychiatric symptoms + toxicity | 1 (2.1%) |
| **Previously treated for TB** | 13 (27.7%) |
| **Co-infection** | |
| Hep B | 1 (2%) |
| Hep C | 5 (10.6%) |

**Virologic outcomes:** Out of the 47 patients on second line cART, 36 (76.6%) were switched due to virologic failure while 11(23.4%) due to toxicity or psychiatric illness/symptoms. Among those patients who were switched to second line due to treatment failure, 34 (94.4%) were analyzed for virologic outcomes, 4 were excluded as they had recently been switched but had not achieved six months on second line cART. Data for two patients was missing for the first 36 months of their second line cART as they had transferred in from other facilities, but their current VL was available, they were included the evaluation of the current VL. Three patients had VL very high due to poor adherence and were not included in this analysis but included the descriptive report (outliers). There was a median (IQR) decrease of VL from 20,022(2208-123845) copies/mL at the time of change to second line cART to 41 (20-220) copies/mL at 6 months of second line cART. The median (IQR) difference of VL at change of cART to second line with VL at 6 months 38,956 (2326-126379) copies/mL.

**Table 2.** Comparison of VL at start and at 6 months on second line cART (all patients) n= 38

| Variable | At start of 2nd -line cART Median (IQR) | At 6 months on 2nd-line cART Median (IQR) | Z- statistica | P-valuea |
|---|---|---|---|---|
| VL (copies/mL) | 20,022 (2208-123845) | 41 (20-220) | - 4.541 | <0.001 |

[a]Wilcoxon Signed Rank test.

A cross-sectional assessment of all patients at the time of the study regardless of the reason for change showed a marked improvement in virologic status with a median (IQR) decrease of VL to 20(20-109) copies/mL compared to the median (IQR) at time of change to second-line cART at 20,022 (2208-123,854) copies/ml. Of these, 77.5% (31/40) had achieved full virologic suppression (VL < 50 copies/mL) and 12.5% (5/40) had adequate viral suppression (VL <400 copies/mL), 2.5% (1/40) had ongoing viremia (VL 400-1000copies/mL) while 7.5% (3/40) with virologic failure (VL>1000copies/mL) at the time of the study. All the three patients with virologic failure had poor adherence and it was the likely reason for the treatment failure.

**Table 3.** Comparison of VL at start of second line cART and current VL (all patients) n = 40

| Variable | At start of 2nd -line cART Median (IQR) | At the time of study 2nd-line cART Median (IQR) | Z-statistica | P-valuea |
|---|---|---|---|---|
| VL (copies/mL) | 20,022 (2208-123845) | 20 (20-109) | -4.372 | <0.001 |

[a]Wilcoxon Signed Rank test

A sub-analysis of patients who had first line treatment failure, out of the 36 patients, 76.6% (23/30) had achieved adequate virologic suppression by 6 months with 47% (14/30) achieving full virologic suppression (<50 copies/mL); with 87% (21/24) of those who had VL testing at 12 months achieving adequate virologic outcome with 58% (14/24) achieving full virologic suppression. At 24, 90% (20/22) of those with VL testing had adequate virologic suppression with 81% (18/22) them having full virologic suppression however 9% (2/22 had virologic failure (>1000 copies/mL). There was a marked VL change at 6 months had a median (IQR) viral load of 78 (20-277) copies/mL compared to that at start of second-line cART 39716(13266-127245) copies/mL.

**Table 4.** Comparison VL at start and at 6 months of second line cART among patients who had first line treatment failure.(n = 30)

| Variable | At start of 2nd -line cART Median (IQR) | At the time of study 2nd-line cART Median (IQR) | Z- statistica | P-valuea |
|---|---|---|---|---|
| VL (copies/mL) | 20,022 (2208-123845) | 78 (20-277) | -4.457 | <0.001 |

[a]Wilcoxon Signed Rank test

**Table 5.** Different levels of VL at start and at 6 months of second-line cART among patient who had first line treatment failure.

| VL copies/mL | At start of 2nd line cART (n=30) | At 6 months of 2nd line cART (n=30) |
|---|---|---|
| < 50 | 0 (0%) | 14 (46.7%) |
| 51-400 | 0 (0%) | 9 (30%) |
| 401-1000 | 2 (6.7%) | 1 (3.3%) |
| >1000 | 28(93.3%) | 6 (20%) |

Adequate virologic outcome (VL<400 copies/mL) was achieved by 76.7% (23/30) of the patients at 6 months of second line cART. Of these, 46.7% (14/30) had achieved full virologic suppression (VL<50 copies/mL). There was 3.3% (1/30) patient with ongoing viremia (VL between 400-1000 copies/ml) and 20% (6/30) patients who had virologic failure (VL>1000 copies/mL) compared to 6.7 (2/30) and 93.3% (28/30) respectively at start of second line cART. Assessment of the subgroup of patients who changed to second line due to toxicity or psychiatric symptoms, 81% (9/11) had viral load at six months and all had adequate virologic suppression. Among them 45% (5/11) were fully suppressed (VL<50 copies/mL). We further assessed the relationship between VL at 6 months with age of the patient using spearman's correlation coefficient as the data did not meet the assumptions for Pearson's Coefficient. There was a small negative correlation between the two variables but, it was not statically significant, r =-0.197, n=44, p = 0.257.

**Immunological outcomes:**

Majority of the patients in our study achieved immunologic success (CD4 cell count >100 cells/mm$^3$) over time. At start of second-line cART, 42.5% (17/40) had immunological failure (CD4< 100 cells/mm$^3$), 10% (4/40) had a CD4 between 100-200 cells/mm$^3$ , 17.5% (7/40) had between 201-350 cells/mm$^3$, 10% (4/40) had between 350-500 cells/mm$^3$ and 20% (8/40) had >500 cells/mm$^3$. There was a median (IQR) CD4 cell count increase to 291(220-497) cells/mm$^3$ compared to the time of change 209 (50-425) cells/mm$^3$. The mean difference of CD4 at

change of cART to second line with CD4 count at 6 months of second-line cART was 107.8 (166) cells/mm$^3$ .

**Table 6.** Comparison of CD4 cell count at start and at 6 months of second line cART (all patients) (n = 38).

| Variable | At start of 2nd -line cART Median (IQR) | At 6 months on 2nd-line cART Median (IQR) | Z-statistica | P-valuea |
|---|---|---|---|---|
| CD4 cell count (cells/mm3) | 203 (50-425) | 291 (220-497) | -3.507 | 0.001 |

[a]Wilcoxon Signed Rank test

A cross-sectional immunologic assessment of all patients on second-line cART at the time of the study showed an increase in CD4 cell count. The median (IQR) CD4 cell count at the time of the study was 461(383-559) cells/mm$^3$ compared to the time they changed to second line cART, 203 ( 50-425) cells/mm$^3$.

**Table 7.** Comparison of CD4 cell count at start of cART to second line and the current CD4 count (n = 40)

| Variable | At start of 2nd -line cART Median (IQR) | Current(latest) 2nd-line cART Median (IQR) | Z-statistic[a] | P-value[a] |
|---|---|---|---|---|
| CD4 cell count (cells/mm$^3$) | 203 (50-425) | 461 (383-559) | -3.700 | 0.001 |

[a]Wilcoxon Signed Rank test

The median score on the CD4 change statistic scale from pre-second line cART (Md = 10.13 ) to 6 months post second line cART (Md = 15.23).A sub- analysis of immunologic outcome was done for patients who had first-line treatment failure as the reason for change to second line by comparing the CD4 count level at the time of treatment change to the level at 6 months and 12 months There was a median (IQR) increase of CD4 count from 150 (50-325) cells/mm$^3$ at change of treatment to 274 (180-411) cells/mm$^3$ at 6 months and at 302 (191-484) cells/mm$^3$ 12 months. There was significant decrease of the number of patients with immunologic failure (CD4 count <100cells/mm$^3$) from the initial 48% (16/33) at the start of second-line cART to 7.4% (2/27) at 6 months and 4.3% (1/27) at 12 months.

**Table 8.** Comparison of CD4 cell count at start and at 6 months of second line cART among those who had first line failure (n = 30)

| Variable | At start of 2nd -line cART Median (IQR) | At 6 months cART Median (IQR) | Z-statistic[a] | P-value[a] |
|---|---|---|---|---|
| CD4 cell count (cells/mm$^3$) | 150 (50-326) | 274 (180-411) | -3.027 | 0.001 |

[a]Wilcoxon Signed Rank test

**Table 9.** Different levels of CD4 count at start and at 6 months of second line cART for those who had first line treatment failure

| CD4 cells/mm3 | At start of 2nd line cART (n=30) | At 6 months of 2nd line cART (n= 27) |
|---|---|---|
| <100 | 13 (43%) | 2 (11.7%) |
| >100 | 17 (56.6%) | 25 (92.5%) |

The patients with immunologic failure (CD4 <100 cells/mm3) 43% (13/30) at start of second line cART compared to 11.7% (2/27) at 6 months of therapy. We further compared the CD4 cell count at 6 months between the males and females. A Mann-Whitney U Test revealed no significant difference in CD4 cell count of males (Md = 15.78, n = 18) and females (Md = 5.08, n = 12), U=103, z = -0.212, p =0.851, r = 0.04. The relationship between CD4 count and VL at treatment at start of second line cART using spearman's correlation coefficient was assessed as the preliminary analysis performed did not meet Pearson correlation assumptions of normality, linearity and homoscedasticity. There was lack of correlation between CD4 cell count and VL at start of second line cART, r =-.411, n=38, p= 0.17. Another relationship analyzed was between CD4 cell count at 6 months and age of the patient using spearman's correlation coefficient. There was no correlation between CD4 cell count and the different ages, r =-0.194, n=44, p = 0.296.

Weight outcomes

This study showed an increase of weight over time compared to the weight at start of second line cART. The median (IQR) weight at start of second line cART was 56 (49-63) compared to 57.5 (50-65) at 6 months. The mean (SD) difference in weight between start and at 6 months of second line cART was 1.8 ± 5kg.

**Table 10.** Comparison of weight at start and at 6 months of second line cART (for all patients) (n = 38)

| Variable | At start of 2nd -line cART Median (IQR) | At 6 months on 2nd-line cARTMedian (IQR) | Z-statistic[a] | P-value[a] |
|---|---|---|---|---|
| Weight (Kg) | 56 (49-63) | 57.5 (50-65) | -2.099 | 0.035 |

[a]Wilcoxon Signed Rank test

A statistically significant increase in weight at 6 months compared to the time of change of cART to second line, z = -2.099, *p*= 0.035, with a small size effect (r = 0.254). The median rank on the weight change statistic scale from pre-second line cART (Md = 13.10) to 6 months post second line cART (Md = 16.70).A cross sectional analysis of weight was done for all patients on second line at the time of the study compared to the time of start of second line cART. The weight at the start of second line cART and the current weight (weight at the time of the study) showed a mean (SD) increase of 3.1kg ±7.6 (95% CI: 0.7- 5.6).

**Table 11.** Comparison of weight at start of second line cART and the current weight at the time of the study (n= 40)

| Variable | At start of 2nd -line cART Median (IQR) | At the time of the study on 2nd-line cART Median (IQR) | Z- statistic[a] | P-value[a] |
|---|---|---|---|---|
| Weight (Kg) | 56 (49-63) | 57 (50-66.8) | -2.274 | 0.023 |

[a]Wilcoxon Signed Rank test

A statistically significant increase in weight at the time of study (current) compared to the time of change of cART to second line, z = -2.274, *p*= 0.023, with a small size effect (r = 0.262). The median score on the weight change statistic scale from pre-second line cART (Md = 15.35) to 6 months post second line cART (Md = 17.72).

Safety outcomes

Adverse drug reactions (ADR) are routinely documented in the patients records as observed by the clinician or reported by the patient. The total number of ADRs that had been reported among patients on second line cART at the time of the study were 36. Dyslipidemia secondary to PIs was the most common occurring in 16 (44.5%) patients followed by gastrointestinal symptoms include nausea, vomiting and diarrhea due to LPV/r occurred in 13 (36%) while hyperbilirubinemia due to ATV/r occurred in 4 (11%) and renal impairment occurred in 1(2.7%) patient. (Table 8)

**Table 12.** Adverse drug reactions

| Toxicity | n (%) | Suspected causative agent |
|---|---|---|
| Dyslipidaemia | 16 (34.5%) | PIs |
| GI symptoms | 13 (27.7%) | LPV/r |
| Hyperbilirubinemia | 4 (8.5%) | ATV/r |
| Acute renal failure | 2(4.2%) | TDF |
| Drowsiness | 2 (4.2%) | TDF/3TC |
| Total ADRs | 39 | |

**HIV Drug Resistance**

Drug sensitivity testing (DST) was carried out in 52% (19/36) patients on second line cART. DST results were available for 47 % (17/36) of the patients with treatment failure. There were additional two other patients who transferred to HTAR while on second line cART and had genotype test from their previous facility of care while one patient had DST but was susceptible to all ART except EFV and NVP which had high level resistance, he was maintained on first line on the same regimen. The three were not included in the analysis. Among the 17 (42%) patients with DST; 11.7% (2/17) had single class mutation, 64.7 % (11/17) had double class mutation while 11.7% (2/17) had triple class mutations (ART classes: NRTIs, NNRTIs, PIs). Two patients (11.7%) had no clinically significant mutations, one had poor adherence and was likely not taking his cART at the time of DST. Overall, 88% (15/17) had at least one resistant mutation.

**Table 13.** ART class mutations

| ART class mutation | n. (%) |
|---|---|
| **No class mutation** | 2 (11.7%) |
| **Single class mutation** | 2 (11.7%) |
| **Double class mutation** | 11 (64.7%) |
| **Triple class mutation** | 2 (11.7%) |

## DISCUSSION

Characteristics of The Study Subjects

In this study of 47 patients on second line cART there were more male (62%) than females (32%). More than 90% of the cases cumulatively are male while females account for less than 10% of the total number (Choy, 2014). These gender difference was also reported in a study in India and eastern Europe with proportion of the male gender ranging between 65-75% of the total population (Chakravarty et al., 2015; Patel, Desai, Shah, & Dikshit, 2013). Most of the study patients were Malays, 40% (19/47) followed by Chinese, 32% (15/47) and Indians, 26% (12/47). The difference may be due to the general proportion of the three ethnicities in Malaysia.

A study conducted in India found that 51% of the patients with first line failure both immunological and virologic failure (Patel et al., 2013). Our study had similar findings with 33 % (13/36) having both combined immunological and virologic failure while some patients had only virologic failure. This may be attributed to the fact that patients failing a regimen have high viral loads. Due to the HIV natural course of destruction of CD4 cells, the amount of CD4 cells will diminish over time resulting in immunologic failure in addition to the virologic failure. On the contrary, several studies have found a lack of correlation between CD4 count and VL during treatment failure. This is because the CD4 count lags behind virologic failure by several

months and may result in delayed diagnosis of treatment failure exposing the patient to increased risk of opportunistic infections and increased morbidity and mortality among other outcomes (Gunda et al., 2017). It likely means that if virologic failure is detected early, the patients may not have reached immunologic failure. It has been shown that the progress to treatment failure varies among individuals with some having non progressive disease (Cao et al., 1995). If CD4 count was used to diagnose treatment failure (CD4< 100 cells/mm3), diagnosis of 47% (17/36) of patients with virologic failure (VL>1000copies/mL) who did not have immunological failure would have been missed or delayed. In our analysis, there was lack of correlations between CD4 and VL (p = 0.17). Patients previously treated for TB were predicted to be 1.5 -3 times at higher risk of first line failure compared to those who have never had tuberculosis (TB). One of the studies had 35% of the patient having been previously treated for TB (Rajasekaran et al., 2007). Patients develop TB are graded as WHO stage 3 (WHO, 2016) where is a progressed HIV infection. The reason for the high first line cART failure rate among patients who had previously been treated for TB may be because patients with low CD4 count are likely to get opportunistic infections like TB. Data from this study showed only 36% (13/36) of the patients who had virologic failure had previously been treated for TB and the most likely cause for the failure was virologic failure secondary to poor adherence.

Virologic Outcomes

Adequate virologic outcome should be achieved between 4-6 months of effective second line cART and then remain suppressed thereafter (WHO, 2016). In this study, 76.6% (23/30) of patient who had first line treatment failure achieved adequate virologic suppression by 6 months (VL <400 copies/ml). These findings suggest that treatment with second-line cART resulted in improved adherence and virologic status of the patients. Several studies have reported successful virologic suppression in patients using second line cART. This results was comparable to a study done in Eastern Europe which showed that the patients who achieved adequate viral suppression at 6,12,24 and 36 months ranged between 79 to 83% during the different period (Chkhartishvili et al., 2014). These results were similar to recent large trial from Africa which showed adequate virologic suppression in 86% of patients on a PI and NRTI based second line regimen at 24 months of treatment (Paton et al., 2014). Another study in India also found statistically significant decrease in viral load with 76% of patients achieving adequate virologic outcome (VL <400 copies/mL) at 6 months and 82% 12 months of second line cART (Patel et al., 2013). A study in China found up to 90 patients achieved adequate virologic suppression rates (< 400 copies/mL) at 30 months after switching to second line cART (Han et al., 2015). On the contrary a metanalysis on second-line treatment outcomes in low-and-middle income countries found virologic suppression to be 77%, 73%, and 38% at 12,24 and 36 months with the decline in the number of patients suppressed over time being attributed mainly to poor adherence (Ajose et al., 2012). The success seen in the early stages of second line maybe a due to intensified follow up and adherence counselling a coupled with a more effective regimen in second line therapy. Poor adherence is a significant risk factor for second line failure and a major risk of death after one year of virologic failure (Chakravarty et al., 2015). In this study patients who reported to have poor adherence 23 % (7/30) also had virologic failure at 6 months of second line cART. A nearly perfect adherence (> 95 %) has widely been stated as the requirement to achieve adequate viral suppression (Bazabhe et al. 2016). This is because, poor adherence results in sub-optimal drug levels in the blood which provides a good environment for viral multiplication and subsequent virologic failure.

Immunologic Outcomes

The CD4 count is a strong predictor of HIV-related complications at any stage of treatment. The adequate immunologic response is defined as an increase in CD4 cell count between 50-150 cells/$mm^3$ during the first year of cART followed by an average increase from 50-100 cells/$mm^3$ per year at a steady state after a year (Nash et al., 2008). This study showed an overall increase of CD4 cell count with mean (SD) increase of 107 (166) cells/$mm^3$ at 6 months. The CD4 change from a median (IQR) of 203 (50-425) cells/$mm^3$ at treatment change to 291 (220-495) cells/$mm^3$ at 6 months of second line cART. These findings suggest that treatment with second-line cART resulted in immunologic reconstitution hence improve of immunologic parameters among the patients in this study. The results were comparable to a large prospective analysis on long term immunologic outcomes of patients in Latin- America, Sub-Saharan Africa, Asia. They suggested that there is progressive increase of CD4 among who remained on therapy. Their study showed a median increase of CD4 cell from 114 cells/$mm^3$ at initiation to 230 cells/$mm^3$ at 6 months,263 cells/$mm^3$ at 12 months , 336 cells/$mm^3$ at 24 months, 372 cells/$mm^3$ at 36 months, 372 cells/$mm^3$ at 48 months and 395 cells/$mm^3$ at 60 months (Nash et al., 2008). Other studies in India, Africa and China showed a median CD4 count increase to between 262- 352 cells/$mm^3$ at 6 months and 394-417 cells/$mm^3$ at 12 months (Cao et al., 2019; Patel et al., 2013). A decline in viral load to < 500 copies/mL is associated with good immunologic outcomes although some patients may have good virologic outcomes but without significant improvement in the CD4 cell count. This was true for this study as all the patients with VL< 500copies had a CD4 cell count >100 cells/$mm^3$ at the time of the study. The immunologic reconstitution can be explained by the fact that when the VL is suppressed there is limited destruction of CD4 cells and hence more CD4 cell can survive for longer duration which results in increase CD4 cell in the body.

Although majority patients achieve an increase in the CD4 cell after change to second line cART, a small proportion of patients delay in achieving adequate immune reconstitution. In some cases, patients may achieve virologic success but fail to have a rise in CD4 cell count. Previous studies have shown that baseline CD4 count is a strong predictor of immunologic response. Patient with very low baseline CD4 cell values before initiating cART were at a higher risk of an impaired CD4 response to cART and most of them had persistently low CD4 cell count after initiation of cART (Brennan et al. 2013). The delay in immunologic response in patients who already have low CD4 count before starting an effective cART regimen means it will take longer for them to achieve adequate CD4 cells. It may also be as a result of the higher viral load the patient had which destroys the CD4 cells and hence affect the amount of CD4 cell in the body.

Weight Outcomes

In this study there was an increase in body weight over time compared to the weight at start of second line cART. The mean (SD) of the difference of weight from start of 2nd line and at 6 months was 1.8 ± 5kg. These results were similar to a study in India which found statistically significant increase in body weight of patients at 6 months and 12 months of second line cART with a mean weight gain at 12 months was 2.2kg (Patel et al., 2013). Generally, uncontrolled HIV is associated with fat loss and poor appetite resulting in weight loss (Grinspoon et al. 2003). Therefore, weight gain among the patients who had previously lost weight may be associated with controlled HIV and treatment success.

Safety Outcomes

In this study dyslipidemia was present in 16 (34.5%) patients followed by gastrointestinal symptoms include nausea, vomiting and diarrhea 13(27.7%) while hyperbilirubinemia 4 (8.5%) and renal impairment 2 (4.2%). A study in India found similar toxicities in their HIV infected patients with dyslipidemia being the most common toxicity (Patel et al., 2013). The increased incidence of dyslipidemia can be explained by the fact that protease inhibitors (PIs) are the main second line agents and dyslipidemia and cause insulin resistance is among the main toxicity associated with the PIs (Overton et al., 2016). Ritonavir boosted PIs has been associated with gastrointestinal symptoms with Lopinavir/r and FPV/r showing the highest rates of these symptoms. This was also demonstrated in our study. with most patients on LPV/r cause all the gastrointestinal symptoms. Anemia is also expected to be high due to the use of zidovudine in second line. Long term use of tenofovir has been associated with decline in renal function and can result in significant impairment of renal function in <5% of patients (Tourret, Deray, & Isnard-Bagnis, 2013). In our study 4% (2/47) developed acute renal impairment secondary to suspected tenofovir toxicity. cART toxicity has been associated to poor adherence. The toxicity may impact on the quality of life of the patients and cause increase morbidity e.g., the development of diabetes or CHF or renal impairment and may result in poor adherence.

**HIV Drug Resistance**

A high rate of resistant mutations was observed among the patients failing first-line cART. Among the 17 (42%) patients with DST; 16.7% (3/17) had single class mutation, 70% (12/17) had double class mutation while 12% (2/17) had triple mutations. Two patients had no clinically significant mutations, one had poor adherence and was likely not taking his cART at the time of DST. Overall, 88% (15/17) had at least one resistant mutation. A large analysis in India found, 78% of the patients failing first line had resistant mutations. Of these, 68% had NRTI resistant mutations and 73% had NNRTI mutations (Karade et al., 2018).

## CONCLUSION

This study showed that second-line cART resulted in good clinical, immunological and virologic outcome in HTAR- ID clinic. Optimal adherence is the cornerstone for success in HIV management. Among the patients who were active in care at the time of the study 90% (40/44) reported to be adherent to their therapies and follow up, they also had adequate virologic suppression. It has been shown that successfully suppressing the VL diminishes the ability of the virus to attack the patient's CD4 cells resulting in increased CD4 cell therefore the immune function is restored and preserved there is reduce HIV associated complications. Therefore, the main goal of treatment is to suppress the plasma viral load maximally and sustainably to below undetectable level. This was achieved in the study as all patients with immunologic failure (CD4 <100 cell/mm$^3$) at start of second line had achieved a CD4 >100 cells/mm$^3$ at 6 months of treatment. Evidence has shown that failing a cART regimen is a strong predictor of likelihood of failing another regimen hence adherence should be emphasized in all stages of care. HIV is an ever-changing virus, at individual and population level; resistant mutation poses a significant challenge in HIV care. These studies emphasize the need for timely drug sensitivity testing to detect and provide early switch to an effective regimen. Some of the patients have resistant mutations to certain antiretroviral drugs, this is inevitable as majority of HIV infected patients will likely require a change of regimen in the course of their lives due to development of resistance. Understanding the profile and resistance patterns is necessary for ensuring effective and long-term survival and help plan for next line of treatment for example third line cART for those patients failing second line cART. Therefore, it is important to monitor patients closely and regularly on second line therapy and address any challenges promptly assess. This can be done more effective through multidisciplinary team as it is done in HTAR-ID clinic.

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