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"TO STUDY THE EARLY CLINICAL, IMMUNOLOGICAL AND VIROLOGICAL PROFILE OF HIV PATIENTS ON SECOND-LINE ANTIRETROVIRAL THERAPY"

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Abstract

Introduction: HIV/AIDS progressively weakens immune system and make it susceptible to life threatening opportunistic infections, neurological disorders or certain unusual malignancies. Second-line ART is the next regimen used in sequence immediately after first-line therapy has failed. Hence, the criteria to switch to second line ART includes immunological and/or virological and/or clinical failure. Manipur is one of the high prevalence states for HIV infection in India. There is so far very little study and data regarding patients in the early phase of second line ART from this part of the country. The present study describes the clinical and immunological outcomes of PLHIV on PI-based second-line ART regimens in north-eastern India thereby identifying the need of change to third line ART, at an early stage. In addition, it determined the WHO clinical staging, CD4 counts and plasma viral load of patients on second line ART at 0 and 6 months and to assess the adherence of patients on second line ART.

Methods: This hospital based longitudinal study enrolled 73 HIV positive patients on second line ART, admitted in Medicine ward, attending Medicine OPD, Center of Excellence (Coe)ART Centre, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur (RIMS) from January 2021 to October 2022. Blood samples were sent for CD4 count, and plasma viral load at baseline and after 6 months on second line ART.

Results: The mean age group was 38.37±9.93 years, majority were males (n=56, 76%), the most common complaints were fever (27.40%), diarrhoea (20.55%) and the common source of infection was heterosexual and IVDU 38% and 32% respectively. The most common regime used was TDF+3TC+DTG (52%, 38). There was improvement in mean BMI {from 21.20(±1.69) to 22.3±1.58 kg/m^2}, mean CD4 cell count (from 266 cells/mm^3 to 440 cells/mm^3), reduction in viral load from172, 892.7(±189,040.8) to 7349.65(±22,526.4), at end of 6 months. Viral components in

blood samples from 33% (24) of study subjects were undetectable at the end of 24 weeks of treatment. Overall adherence to ART among study subjects was good (90.41%) and the association between treatment adherence and effect of second line ART for 6 months was found to be statistically significant.

Conclusion: The present study revealed improvement in CD4 count, significant virological suppression and immunological recovery in patient started on second line ART following failing first line ART, who were followed for 6 months. Thus, strict adherence to the national policies on HIV testing, proper management of positive clients and full implementation of the "Treat All" policy, could help achieve, if not all, but at least the "95, 95, 95" target by 2024.

Keywords: *HIV/AIDS, HAART, second line ART,CD4 count, viral load*

INTRODUCTION

In India, the adult HIV prevalence estimated (15-49 years) was 0.21% in 2021. The largest burden of the disease is shared by north-east region states. Mizoram's prevalence was 2.70%.Nagaland had 1.36% and Manipur accounts for 1.05.The number of People Living with HIV (PLHIV) was around 24 lakhs.[1]

Second-line Anti-Retroviral Therapy (ART) is the regimen used next immediately following the failure of first-line ART. The National AIDS Control Organization (NACO), Government of India, in August 2008, started free second line ART followed by launch of third line ART in June 2016.The national guideline recommended second line regimen as one new nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) plus Lamivudine plus one protease inhibitor [either Atazanavir (ATV) or Lopinavir (LPV) boosted by Ritonavir (RTV)]. Highly active antiretroviral therapy, or HAART introduced in 1996 is defined when several such drugs, typically 3 or 4, are taken in combination to prevent developing resistance.[2]One should arouse suspicion of second line failure when viral load >1000 copies/cu mm at 6months of initiation and CD4 count less than baseline of second line initiation, more than 50% drop from the highest value during the treatment, less than 100/cu mm, as per NACO. If viral load is 1000 or more repeat the viral load after 3months. Failure is defined if viral load >1000/cu mm.[3]At end of 2019, there are 38 million PLHIV in the world, out of which 67% of them had access to ART.[4]Approximately, 3 million PLHIV will receive second-line, boosted PI-based ART by 2020.[5] The criteria to switch to second-line ART includes immunological and/or virological and/or clinical failure.[6]

There was significant improvement in the immuno-virological response with 2nd-line ART which was considered to be effective and reliable with as per Modi JP et al[7](84.12% of patients had improvement in their mean CD4 count), Ferradini et al[8], Singh J et al[3]and Achappa B et al[9]. There was statistically significant association of virologic success with good adherence, high baseline CD4, and early centre for Disease Control and Prevention (CDC) staging ($P<0.05$). While Thao VP et al reported that treatment failure and significantly shorter time to second-line failure was associated with older age, high baseline viral load ,history of IVDU, lower CD4 count, medication adherence <95%, and use of a protease inhibitor other than lopinavir or atazanavir [10]. In a resource-limited setting, second line ART based on the genotype testing results yielded good virologic and immunologic outcomes, and there was scaling-up of second-line ART. Adherence improved after second-line ART switch as per Murphy RA et al[11]. Outcome data in Cambodia suggested a high rate of virological suppression and immune reconstitution with $LPV_{/r}$-based 2nd-line regimen after follow up of 24 months. In the presence of ART, HIV rapidly acquires resistance mutations and ability to replicate. In resource-limited settings, the rate of virological failure on 2nd-line therapy are high (limitation to the access to treatment options beyond 2nd-line) and associated with poor adherence rather than drug resistance along with duration of exposure to previous drug regimens. Madec Y et al in his study concluded that higher viral load at 2nd-line initiation is indicative of added drug

resistance and poor adherence to ART [12].ART improves wasting and other acute parameters of nutritional status, clinical outcome, and restores the immune system. At present first-line ART is effective in HIV-positive children in Choudhary N etal[13].Authors concluded, as combination therapy with multiple drugs can potently and durably suppress HIV-1 replication in vivo. Standard ART appeared to prevent permanent loss of important subsets of CD4 T lymphocytes, particularly when treatment preceded the antibody response to HIV in Zaunders JJ et al[14].Ciaffi L et al mentioned in his study, that the most important prognostic factor for successful second-line treatment was high viral load [15].

The rate of HIV replication largely determines the rate of decline in CD4 count.[16] Antiretroviral therapy (ART) improves immunological response and hence leads to substantial decrease in plasma HIV RNA levels, thereby reversing the downward trend in CD4 lymphocyte cell counts.[17-20]The median time of progression from HIV infection to AIDS in the absence of ART is 9-10 years, and after developing AIDS, the median survival time is only 9.2 months.[21]

Adherence to ART has been cited as a major factor associated with poor outcomes among those factors affecting the ART outcome[22-24] and greater than 95% adherence has been suggested for maximum effect.[25-26]This will help us in identifying the need to boost and enhance the counselling sessions for better adherence to get a good outcome in patients with second line ART and hence minimise the need for third line ART.

North-eastern states, Nagaland, Mizoram and Manipur have a high prevalence, greater than the national prevalence for HIV[27]. Manipur is one of the high prevalence states for HIV infection in India. There is so far very little study and data regarding patients in the early phase of second line ART. About 3% of patients on first-line therapy fail the regimen annually and need switch to protease inhibitor-based second-line ART for survival [3]. The present study describes the clinical and immunological outcomes of PLHIV on PI-based second-line ART regimens in north-eastern India thereby identifying the need of change to third line ART, at an early stage.

MATERIALS AND METHODS

This hospital based longitudinal study enrolled 73 HIV positive patients on second line ART, admitted in Medicine ward, attending Medicine OPD, Center of Excellence (Coe)ART Centre, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur (RIMS) from January 2021 to October 2022.

Inclusion criteria included HIV positive patients 18 years of age or older on second line ART at the time of initiation and followed up at 6 months.

Exclusion criteria included terminally ill patients, those with co-morbid conditions (Sepsis, Renal failure, liver failure, etc), those with chemotherapy/malignancy, patients with major psychiatric illness and those not giving consent.

Study Procedure: All the eligible patients were subjected to comprehensive questionnaire/ history taking/ thorough detailed examination including clinical staging, as per NACO recommendation. A proper proforma was recorded with age, ART drug history, clinical staging and BMI,CD4 count, HIV plasma viral load and adherence to ART. Blood samples were sent for CD4 count, plasma viral load at baseline and after 6months on second line ART. A total confidentiality was maintained by coding of patient's data throughout the study. Follow-up and monitoring were done to track clinical progress, adherence, and to monitor lab values of CD4 counts and plasma viral load at baseline and at 6 months.

Study tools:

Immunological monitoring:

CD4 testing: CD4 Counts (as per NACO guidelines, by Becton, Dickinson and company (BD) Fluorescent Activated cell Sorter # Count™ (FACSCount) System, at baseline and 6 months of starting second line ART.

Virological monitoring: Plasma Viral load by Abbott Real Time HIV-1 assay (carried out by Abbott® m2000 system) uses RT-PCR technology with homogenous real-time fluorescent detection, at baseline and 6 months after initiation of second line ART, at HIV-1 VL testing laboratory of National Reference Laboratory (NRL) at Department of Microbiology, RIMS, Imphal, Manipur.

WHO Clinical staging and clinical monitoring - Clinical staging of the patient was done at baseline and after 6 months as per WHO guidelines based on body weight, overall well-being, any new symptoms / signs, four symptom screening for TB at every visit, treatment Adherence-Evaluation- Pill count, self-reported adherence and adherence to ART was assessed at each visit as per NACO guidelines 2021 and reinforced through counselling at each visit .

General	Record vital signs, body weight, height and body mass index (BMI), temperature, blood pressure, pulse rate, respiratory rate, pallor & icterus
Appearances	Unexplained moderate or severe weight loss, HIV wasting • Rapid weight loss is suggestive of active Opportunistic Infections, especially if associated with fever • Gradual weight loss (not caused by malnutrition or other obvious illness) is suggestive of HIV infection • "Track marks" and soft tissue infections which are common among IDUs
Consider conditions other than HIV	• Malaria, Tuberculosis, Syphilis, Gastrointestinal Infections, Bacterial Pneumonia, Pelvic Inflammatory Disease, Viral Hepatitis other than HIV
Skin	• Signs of HIV-related and other skin problems. These include diffuse dry skin, typical lesions of PPE, especially on the legs, Seborrhoeic Dermatitis on the face and scalp • Herpes Simplex and Herpes Zoster or scarring of previous Herpes Zoster (especially multi-dermatome)
Lymphnodes	• Posterior cervical nodes • PGL (Persistent Glandular Lymphadenopathy) typically presents as o Multiple bilateral, soft, non-tender, mobile cervical nodes, other than axillary or inguinal nodes • Tuberculous lymph nodes typically present with constitutional symptoms such as fever, night sweats and weight loss
Mouth	• Signs suggestive of HIV infection including white plaques on tongue, cheeks and roof of mouth (oral candida), white striped lesions on the side of the tongue (OHL) and cracks at the corners of the mouth (Angular Cheilitis) • Difficulty in swallowing is commonly caused by oesophageal candida
Chest	• The most common problems are TB, CAP and PCP • Signs and symptoms are cough, shortness of breath, haemoptysis, weight loss / poor weight gain in children, fever, night sweats, congestion or consolidation • Perform a chest X-ray PA view
Abdomen	• Hepatosplenomegaly, masses and local tenderness
Neurological	• Perform comprehensive neurological examination. • Fundus examination if CD4 less than 100
Ano genital	• Herpes Simplex and other genital sores / lesions, vaginal or urethral discharge; perform PAP smear

Statistical analysis:

STATA version 16.0, Stata Corp, Texas, USA was used for analysis of data. Quantitative variables were presented in terms of mean (± standard deviation) (Mean±SD). The Wilcoxon signed-rank test was used to compare the values at initiation and at 6 months respectively. The Fisher's exact test was applied to determine the association between treatment adherence and effect of second line ART for the duration of 6 months. A p-value <0.05 was considered statistically significant.

Approval of Research Ethics Board and Informed Consent The study was approved by Research Ethics Board Regional Institute of Medical Sciences, Imphal (REB No: A/206/REB – Comm (SP)/RIMS/2015/708/50/2020)

RESULTS

A total of 73 patients were enrolled in this study after informed consent. Baseline characteristics were shown in table I. The mean age group was 38.37±9.93 years, majority were males (n=56, 76%) while females were 23 (17%), the most common complaints were fever (27.40%),diarrhoea (20.55%) followed by headache, malaise, cough, dysphagia and constipation. The common source of infection was heterosexual and IVDU 38% and 32% respectively followed by vertical transmission (12.33%), blood/blood products transmission (10.96%) and needle stick injury (5.48%). The mean BMI of the participants was 21.20(±1.69) which increased to 22.3±1.58 kg/m^2 at end of 6 months. Comparison of ART regime and WHO clinical staging at baseline and after 6 months were given in table II.TDF+3TC+DTG (52%,38) was the most common regime used. According to WHO clinical staging, majority (52%,38) belonged to stage 2 which improved to stage 1 (53.4%,39) after 6 months ART. During the treatment course, 4 patients (5.48%) deteriorated, 4 (5.48%) of them remain the same while 65 (89.04%) improved and 3 patients (4.11%) expired. Overall adherence to ART among study subjects was good (90.41%) while 9.59% were poor and the association between treatment adherence and effect of second line ART for 6 months was found to be statistically significant (p-value: <0.001).Mean difference of BMI, CD4 count and viral load at baseline and after 6 months of ART were shown in table III. The mean CD4 cell count improved from baseline value of 266 cells/mm^3 to 440 cells/mm^3 at 6 months. Second-line ART acted notably on the positive direction in terms of reducing the viral load of HIV. The baseline mean viral load was 172,892.7 (±189,040.8) which reduced to 7349.65(±22,526.4) at the end of the 6 months treatment. Viral components in blood samples from 33% (24) of study subjects were undetectable at the end of 24 weeks of treatment. Proportion and association of treatment effect on treatment adherence were given in table IV where there was statistically significant association (p value <0.001) between improvement in the effect of treatment with good adherence to ART.

TableI. Baseline characteristics of the study subjects(N = 73).

Parameters	**Results n(%)73 (100%)**
Age in years, mean (range)	38.37±9.93
Gender: Male	56(76.71%)
Female	17(23.29%)
Symptoms	
Fever	20(27.40%)
Diarrhoea	15(20.55%)
Headache	12(16.44%)
Malaise	11(15.07%)
Cough	9(12.33%0
Dysphagia	2(2.74%)
Constipation	1(1.37%)
No complaints	3(4.11%)
Sources of HIV transmission	
Sexual transmission	28(38.36%)
Intravenous drug use (IVDU)	24(32.33%)
Vertical transmission	9(12.33%)
Blood /blood products transfusion	8(10.96%)
Needle stick injury	4(5.48%)

Table II. Comparison of different parameters at baseline and after 6 months(N=73).

Baseline			After 6 months of ART		
ART regime	Frequency	Proportion	ART regime	Frequency	Proportion
AZT+ 3TC+EFV	2	2.74%	ABC+3TC+DTG	3	4.11%
AZT +3TC+NVP	3	4.11%	ABC+3TC+LPV/r	1	1.37%
TDF+3TC+NVP	6	8.22%	TDF+3TC+ATV/r	17	23.29%
TDF+3TC+DTG	38	52.05%	TDF+3TC+DTG	28	38.36%
TDF+3TC+EFV	24	32.88%	TDF+3TC+EFV	2	2.74%

			TDF+3TC+LPV/r	22	30.14%
WHO clinical stage					
Stage 1	1	1.37%	Stage 1	39	53.42%
Stage 2	38	52.05%	Stage 2	28	38.36%
Stage 3	30	41.10%	Stage 3	3	4.11%
Stage 4	4	5.48%	Expired	3	4.11%

Abbrevations -T- tenofovir, L/TC- lamivudine, D- dolutegravir ,A- abacavir, Z-zidovudine, E-efavirenz,N- nevirapine, LPV – lopinavir, r - ritonavir

Table III. Mean difference of different parameters at baseline and after 6 months of ART(N=73).

Parameters	Baseline	After 6 months	P – value(Wilcoxonsigned-rank test)
BMI (kg/m2)	21.20(±1.58)	22.35(±1.58)	<0.001
CD4count (cells/mm3)	266.48(±72.76)	440.44(±108.49)	<0.001
Viral load (copies/ml)	172,892.7(±189,040.8)	7,349.65((±22.526.4)	<0.001

Table IV: Proportion and association of treatment effect on treatment adherence(N=73).

Treatment Adherence	Effect of treatment			Fisher's Exact test
	Improved	Remained same	Deteriorate	p-value
Good	65 (98.48%)	1 (1.52%)	0	<0.001
Poor	0	3 (42.86%)	4 (57.14%)	

DISCUSSION

A total of 73 HIV positive patients who were started on second line ART at the time of enrolment constituted the study population; they came with a wide range of disease stages, blood parameters and symptoms. The patients were mostly male (76.71%) and middle aged (38.37 ±9.93 years). Most of them reported with fever (27.40%) or diarrhoea (20.55%). Major sources of transmission among the study populations were Intra-venous drug use (IVDU)(32.88%) or sexual transmission (38.36%).

Second-line regimen for HIV positive patients composed of a boosted protease inhibitor (bPI), such as ATV/r and LPV/r, and or INSTI (Integrase strand transfer inhibitors) (Dolutegravir/DTG)[27] along with two nucleoside analogues (NRTIs)[28]. The rationale for the selection of the NRTIs in second-line therapy is to choose the most appropriate combination depending on what was used in the first-line regimen.The second line of drugs are chosen based on two clinical scenarios: 1. early switching based on sensitive monitoring for failure, using viral load; 2. late switching based on insensitive monitoring, using clinical or immunological criteria for defining failure.

The enrolled patients from this study reported with high prevalence of IVDU, nearly 33% and sexual transmission (38.36%). Sehgal P N et al[29] estimated in 1991, that 23.1% of nation's HIV cases were contributed by IVD users of Manipur. Singh AK et al[30] in his study concluded that, HIV seropositivity rate was almost 10 times higher than the overall rate for India, in the state of Manipur. "Primary HIV infection" as coded by Apoola Aetal[31]included pyrexia, pharyngitis, malaise, lethargy, maculopapular rash, mucous membrane ulceration, lymphadenopathy and headache; few of them were present among our study participants. In our study, majority patients reported with fever (27.40%) or diarrhoea (20.55%).Among others diarrhoea[32] and fever[33] were also found to be frequent complications associated with HIV-infected patients.

At the end of treatment for 6 months median BMI of the participants increased by 1.15 kg/m2 as compared to baseline. Baraki AG et al[34] described, for a 1-month increase in treatment duration, the BMI of patients increases by 0.04 kg/m^2, which was further backed by another study among North Indian populations[35].

The median CD4 T-cell count among our study population increased by 140 cells/mm^3 which is consistent with the study by Mugo CW et al[36]. Initially most patient's logarithm CD4 count increased rapidly and then stabilized. Authors also discussed the growth in CD4 count varied due to different regime of medicines during the second-line ART. They explained after initiation to ART the rise of logarithm CD4 count in patients on Efavirenz is faster compared to those on Nevirapine.
Among the study population, second-line ART acted notably on the positive direction in terms of reducing the viral load of HIV. The median viral load at baseline was 110,000 copies/ml, that reduced to 1490 copies/ml at the end of the 6 months treatment. Another significant finding was, viral components in blood samples from 33% of the patients (24 of 73 participants) was undetectable at the end of 24 weeks of treatment, which is consistent with findings by Ferradini L et al[8](85.7%).There could be two probable reasons for such discrepancies: different geographical populations, individual's immunity (innate, as well as acquired)[37], participants' living environment, mother's health at birth, nutritional and immunization status, presence or absence of any communicable or non-communicable diseases and such many other associating physical, environmental factors.

Among the participants of our study more than 90% of the patients adhered to the drug regime which is similar to study from South-Western Ethiopia, an African country (83.3%). Those who maintained the treatment regime, all of them, except one, was found to be improving their health gradually. Similar finding was backed by Gardner EM et al[38], where the authors concluded "excellent adherence to antiretroviral therapy results in marked improvements in a variety of outcomes: better immunological response to therapy, decreases in hospitalizations and emergency room visits, and improved survival"[39,40].

CONCLUSION

The present study revealed significant virological suppression, improvement in mean CD4 cell count and immunological recovery in patient started on second line ART following failing first line ART, who were followed for 6 months. Viral components in blood samples from 33% of the patients were undetectable at the end of 24 weeks of treatment. Thus, strict adherence to the national policies on HIV testing, proper management of positive clients and full implementation of the "Treat All" policy, could help achieve, if not all, but at least the "95, 95, 95" target by 2024.Among the participants of the study more than 90% of the patients adhered to the drug regime, as per NACO guidelines. However further studies are needed that can include bigger sample size in order to acquire the true scenario of virological suppression among HIV patients on ART, thus also be able to detect virological, immunological failure as early as possible.

Declarations:
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Ethical Approval: The study was approved by the Institutional Ethics Committee.

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