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Clinical and immunological profile of patients referred to adult ART plus center for second line ART

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Abstract

Background: Resistance to first-line anti-retroviral therapy (ART) has been a major concern in people living with HIV (PLHIV), which necessitates a switch to second-line therapy. The present study was undertaken to assess the clinical and immunological profile of the patients who were referred for second line ART.

Method: Total 100 HIV positive patients who were referred to adult ART plus center for second line ART and been on first line ART for a minimum duration of 1 year were studied and outcome of ART was monitored using different criteria's including virological, immuno logical, and clinical evaluations.

Results: Out of 100 patients, majority (99%) were HIV-1. Age and gender do not significantly affect the ART/ treatment failure (clinical, immuno logical, and virological) but marital status was associated with the virological failure.

Duration of follow up of ART >7 years was associated with treatment failure. There was significant fall in CD4 at presentation and 6 months before referral to DACEP for second line ART. ADR to first line ART includes Efavirenz induced rash (16.7%), Tenofovir induced nephrotoxicity (33.3%) and Zidovudine induced anemia (50%). Most prevalent opportunistic infection was tuber culosis (20%). Only 10% subjects were poorly adherent to ART. Clinical failure sensitivity- 27.5%, specificity- 77.8% and immuno logical failure- sensitivity- 89.1%, specificity- 33.3%. Initial adult regime with Tenofovir based was significantly associated with virological failure.

Conclusion: Non-adherence is most important predictor of treatment failure. Sensitivity and specificity of clinical/ immuno logical failure alone in predicting virological failure was poor. This highlights the need for considerations of all parameters including viral load (gold standard) before declaring virological failure.

Keywords: Antiretroviral therapy; HIV; Virological; Immunological; CD4; Efavirenz; Tenofovir; Zidovudine

Introduction

India has a low HIV prevalence of 0.22 %. Even with this low prevalence, in terms of absolute numbers, India has

the third highest burden of HIV in the world with an estimated 2.14 million people living with HIV (PLHIV), 87,000 estimated new infections and 69,000 AIDS-related deaths annually [1].

Although antiretroviral therapy (ART) does not cure HIV infection, the decrease in the viral load and the improvement in immunological status brought about by the use of these drugs have resulted in a marked decrease in the mortality and morbidity associated with the disease [2]. Moreover, the use of highly active antiretroviral therapy (HAART) has improved the survival of patients infected with HIV, slowing the progression of the infection towards AIDS. This is achieved through an Immuno logical recovery with increase of CD4 t-cell count and decrease in viral load to undetectable levels [3, 4]. Viral load and CD4 t-cell counts are the most commonly used parameters to monitor the efficiency of antiretroviral treatment. Because of the association observed between immunologic and viral responses in the first months of treatment, it is a common practice to follow-up these parameters to determine therapeutic success or failure and evaluate changes in antiretroviral treatment in early stages [5].

According to recent guidelines of NACO, virological failure is the most sensitive indicator of failure. CD4 helps in suspecting treatment failure but viral load is decisive in switching over to second line of regimen. Considering limited availability of laboratories determining viral load, CD4 forms the mainstay at least to screen first line ART unresponsive patients [6].

In HIV-Infected individuals on ART, the decision on when to switch from first line to second-line therapy is critical. If the decision is made too early the months or years of potential further survival benefit from any remaining first-line effectiveness is lost; if it is made too late, the effectiveness of second-line therapy may be compromised and the patient is put at additional risk of death. Virological monitoring should be used to establish the optimum first-line therapy and time for regimen switching [7, 8]. The cause of failure is an important factor before deciding to modify ART regimen. Nonadherence being the major cause of drug resistance, looking at lifelong treatment and its side effects. In addition, continuance of high-risk activities, which predispose to superinfection by drug resistant virus, should be addressed too. Identification of resistance mutations by genotype testing could further aid appropriate sequencing and rational use of antiretroviral medication and information on resistance rates associated with antiretroviral treatment will help in reducing rate of failure [9]. The present study was undertaken to assess the clinical and immunological profile of the patients who were referred to ADULT ART plus center for second line ART.

Materials and Methods

After obtaining Institutional Ethical Committee and review board approval as well as valid informed consent from all the patients, this prospective/ retrospective observational study was conducted in the Department of Medicine, at Tertiary Care center over a period of 18 months. Our tertiary care center was permitted and supported by MDACS (Mumbai district AIDS control society) to start Adult second line ART referral (DACEP-District AIDS Clinical Expert Panel) dated 12th October 2017. Our center (DACEP) serves as a referral center for 6 peripheral center and also our own tertiary care center. Request for consultation is online and in response to that our DEO (Data Entry Operator) gives the appointment within 2-4 weeks. Subjects referred to DACEP include:

- Suspected clinical and immunological failure

- Severe ADR (adverse drug reaction) to existing ART drugs to consult for change of regime/ use of substitute drugs
- To change regime in case one or more drugs in existing regime is withdrawn under National guidelines.

Study included HIV positive patients who were referred to adult ART plus center for second line ART and been on first line ART for a minimum duration of 1 year and who needed admission in medical wards due to clinical reasons. Mumbai has a large multi-ethnic population, which also reflects in the patients attending our OPD, and so was our study population. The study was conducted only on follow up outpatients. Patients unwilling to give consent for participating in the study were excluded.

Methodology

A total of 100 patients were selected for the study. HIV sero status had been confirmed, using the NACO (Government of INDIA) guidelines. CD4 counts were obtained for each subject using a flow cytometry analysis of peripheral venous blood. Demographic, relevant clinical and laboratory data were obtained by retrospective chart review. Data included age, sex, concomitant sexually transmitted infections and various risk factors such as Hepatitis B and C co-infection and exposure variables of current CD4 cell count, documented baseline CD4 cell count, length of time since diagnosis, ART, other basic laboratory work up such as hemogram, liver and renal function tests, electrolytes. Demographic data, medical history and physical examination and investigations were documented using a pretested proforma. Confidentiality was maintained and there were no conflicts of interests.

Subjects CD4 counts were available under NACO facilities. In all subject's HIV RNA viral load was made available free of cost under NACO guidelines and Public Private Partnership project with a leading National laboratory funded by MDACS through NACO.

Viral load assays quantify the amount of HIV-1 RNA circulating in the blood of an infected individual. The usual measurement of viral load is that of cell free virus in plasma. The available viral load cannot detect copy numbers below 20/ml. The cut off for Virological failure is 1000 copies/ml as per recent guidelines of NACO.

Data Analysis and Interpretation

Data was entered into Microsoft Excel (Windows 7; Version 2007) and analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows software (version 22.0; SPSS Inc, Chicago). Descriptive statistics such as mean and standard deviation (SD) for continuous variables, frequencies and percentages were calculated for categorical Variables. Paired t Test was used to compare CD4 Count over Time. Level of significance was set at 0.05.

Result and Analysis

Out of 100 HIV positive patients, majority (99; 99%) were HIV 1 but we encountered single case of HIV 2 (1%) during the study period. Mean age of patients was 39.95±11.60 years, ranged from 13-69 years with male predominance (56%). Majority (68%) were married at present. Heterosexuality was more common risk factor accounting 92%. The mean duration of ART among the study group was 7.51±4.08 within the range 2-19 years. Maximum i.e., 40% subjects were on ART in the last 6-10 years. 57% subjects presenting to ART plus center were WHO clinical stage 1 as per NACO guidelines and WHO Criteria. However, out of 100, 27% subjects had clinical failure, 87% had immunological failure and almost 91% subjects had virological failure. Total 12 subjects of 100 were having ADR to first line ART. The mean viral load was 91849±159157, ranged from 989-708711. Among 100, 3% subjects were poorly adherent

(<85%) to treatment while 7% subjects with fair good adherence to ART, (Table 1).

adherence (85-95%) and remaining 90% subjects shows

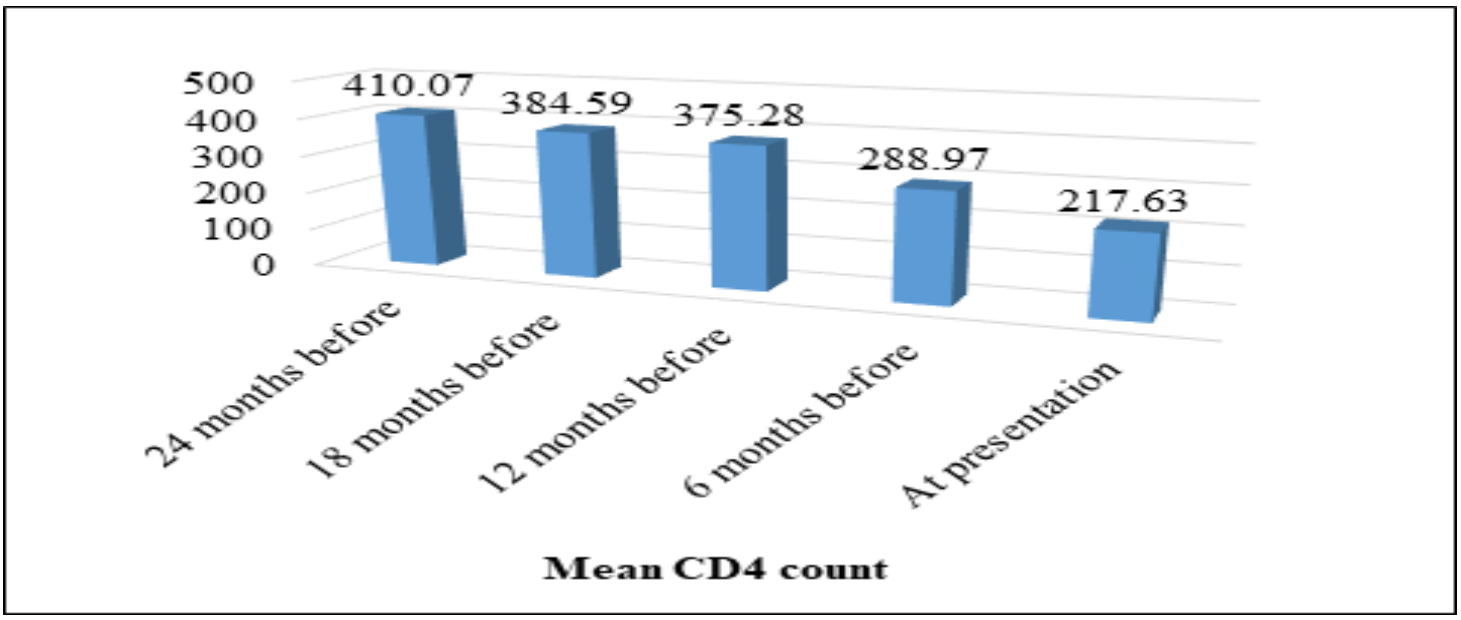
Table 1: Baseline data for eligible patients (N = 100)

Parameters			Frequency	Percentage
Age (in years)	≤30		23	23.0
	31-40		33	33.0
	41-50		24	24.0
	51-60		15	15.0
	>60		05	5.0
Gender	Male		56	56.0
	Female		42	42.0
	Transgender		02	2.0
Marital Status	Married		68	68.0
	Divorced		21	21.0
	Widow		07	7.0
	Single		04	4.0
Risk Factors	Heterosexual		92	92.0
	Mother to Child		06	6.0
	Homosexual		02	2.0
Duration of ART (in Years)	≤5		39	39.0
	6-10		40	40.0
	>10		21	21.0
WHO Clinical Staging	1		57	57.0
	2		17	17.0
	3		19	19.0
	4		07	7.0
Reason for referral to DACEP	Clinical failure		27	27.0
	Immunological failure as per CD4 criteria		87	87.0
	Virological failure with VL>1000 copies/ml		91	91.0
	ADR analysis to ART (n=12) (ADR outline)	Efavirenz induced rash	02	16.7
		Tenofovir induced nephrotoxicity	04	33.3
		Zidovudine induced anemia	06	50.0
		Gynecomastia	00	0.0
	Hyperbilirubinemia secondary to Atazanavir*		02	16.7
Viral Load	<1000/TND		09	9.0
	≤ 10,000		30	30.0
	10,000-1 Lakh		30	30.0
	>1 Lakh		24	24.0
Adherence	70-80%		03	3.0
	81-90%		04	4.0
	91-95%		03	3.0
	96-100%		90	90.0

*After switching to Second line ART 2 patients came for follow up with deranged LFT (Hyperbilirubinemia)

Figure 1 show the decline in mean CD4 counts prior to referral to DACEP, was significant at presentation ($p<0.001$) and 6 months before referral ($p<0.001$).

Figure 1: Mean CD4 counts of study subjects (N=100)



Most prevalent opportunistic infection was pulmonary and extra pulmonary tuberculosis (20%) along with oral candidiasis (10%). Also, there were cases of CMV retinitis, pneumocystis carinii pneumonia (PCP), chronic diarrhea, syphilis and molluscum contagiosum as shown in table 2.

Table 2: Opportunistic infections (OI) in subjects with clinical failure (n=27/100)

Opportunistic Infections	Overall (N=100)	OI in subjects with Clinical failure (N=27)	
		Current OI	History of OI
Pulmonary TB	20	04	16
Tubercular pleural effusion	02	02	00
MDR Pulmonary TB	01	01	00
Abdominal Kochs	02	00	02
Disseminated Kochs	03	03	00
CNS Tuberculoma	01	01	00
TB Lymphadenitis	01	01	00
Oral Candidiasis	10	10	00
CMV Retinitis	01	01	00
Chronic Diarrhoea	01	01	00
PCP	01	01	00
Herpes Zoster	01	01	00
Molluscum Contagiosum	01	01	00
Syphilis	01	01	00

Maximum i.e., 55% of referred subjects were on TLE followed by 41% subjects were on ZLN whereas majority 32% referred subjects were switched to TL + ATA/rit, 29% were switched to ZL + ATA/rit and 18%

were switched to ABC/LAM + ATA/rit as per NACO guidelines as shown in table 3.

Table 3: Distribution of study subjects according to the regime (N=100)

Parameters		Frequency	%
Current Regime	TL+ Lopinavir /RIT	01	1.0
	TL+NVP	03	3.0
	TLE	55	55.0
	ZLN	41	41.0
Switched Regime	ABC/LAM+ATA/RIT	18	18.0
	ABC/LAM+EFV	01	1.0
	ABC/LAM+LOP/RIT	01	1.0
	TL+ATA/RIT	32	32.0
	TL+LOP/RIT	01	1.0
	TLE	05	5.0
	ZL+ATA/RIT	29	29.0
	ZLN	02	2.0
	Referred to SACEP*	01	1.0
*Higher tertiary care referral center for 3rd line ART			

From the table 4, it was observed that age, sex, risk factors and duration of ART were not significantly related to virological failure (p>0.05) while marital status significantly correlated with virological failure (p = 0.015), (Table 4).

Table 4: Association between baseline parameters and virological failure (N=100)

Parameter		Virological Failure		P value
		Yes n (%)	No n (%)	
Age (years)	Mean	39.99 (11.94)	39.56 (7.66)	0.915
Gender	Female	37 (88.1)	5 (11.9)	0.648
	Male	52 (92.9)	4 (7.1)	
Marital Status	Single	2 (50.0)	2 (50.0)	0.015
	Married	62 (91.2)	6 (8.8)	
	Divorce	21 (100.0)	0 (0.0)	
	Widow	6 (85.7)	1 (14.3)	
Risk Factors	Heterosexual	83 (90.2)	9 (9.8)	0.651
	Mother to child	6 (100.0)	00 (0.0)	
	Homosexual	2 (100.0)	00 (0.0)	
Duration of ART	Mean	7.57 (4.17)	6.89 (3.14)	0.635

The association of CD4 count with clinical failure was quite significant with p value= <0.001; 24 months before, 18 months before and 6 months before referral. Also, p value= 0.003; 12 months before and p value= 0.001 at presentation was significant. While association of CD4 count with immunological and virological failure was not significant as clinical failure considering CD4 levels at presentation, 6 months before, 12 months before, 18 months before and 24 months before as shown in table 5.

Overall, it was found CD4 count reduction was significantly associated with clinical failure rather than immunological and virological failure.

Table 5: Association of CD4 count with clinical, immunological, and virological failure (N=100)

Clinical Failure						
		24 months before	18 months before	12 months before	6 months before	At presentation
CD4 Count	<100	8 (72.2)	6 (85.7)	05 (62.5)	7 (77.8)	10 (66.7)
	100-250	11 (44)	11 (40.7)	11 (45.8)	14 (35)	14 (26.9)
	250-500	03 (10)	6 (16.7)	08 (19)	5 (12.5)	03 (10)
	>500	05 (14.7)	4 (13.3)	3 (11.5)	1 (9.1)	00 (0.0)
	P value	<0.001	<0.001	0.003	<0.001	0.001
Immune Failure						
CD4 Count	<100	11 (100.0)	7 (100.0)	8 (100.0)	9 (100.0)	14 (100.0)
	100-250	21 (84.0)	26 (96.3)	22 (91.7)	35 (87.5)	47 (90.4)
	250-500	23 (76.7)	28 (77.8)	35 (83.3)	34 (85.0)	23 (76.7)
	>500	32 (94.1)	26 (86.7)	22 (84.6)	9 (81.8)	3 (100.0)
	P value	0.102	0.121	0.515	0.624	0.227
Virological Failure						
CD4 Count	<100	10 (90.9)	6 (85.7)	6 (75.0)	8 (88.9)	14 (93.3)
	100-250	25 (100.0)	24 (88.9)	23 (95.8)	37 (92.5)	48 (92.3)
	250-500	25 (83.3)	33 (91.7)	38 (90.5)	35 (87.5)	26 (86.7)
	>500	31 (91.2)	28 (93.3)	24 (92.3)	11 (100.0)	3 (100.0)
	P value	0.201	0.895	0.354	0.605	0.755

Association of virological failure with clinical failure was not statistically significant with p value of 0.735. Sensitivity of clinical failure predicting virological failure was 27.5% with specificity of 77.8%. Also, the association of immunological failure with virological failure was not statistically significant with p value of 0.057. Sensitivity of immunological failure predicting virological failure was 89.1% with specificity of 33.3%, (Table 6).

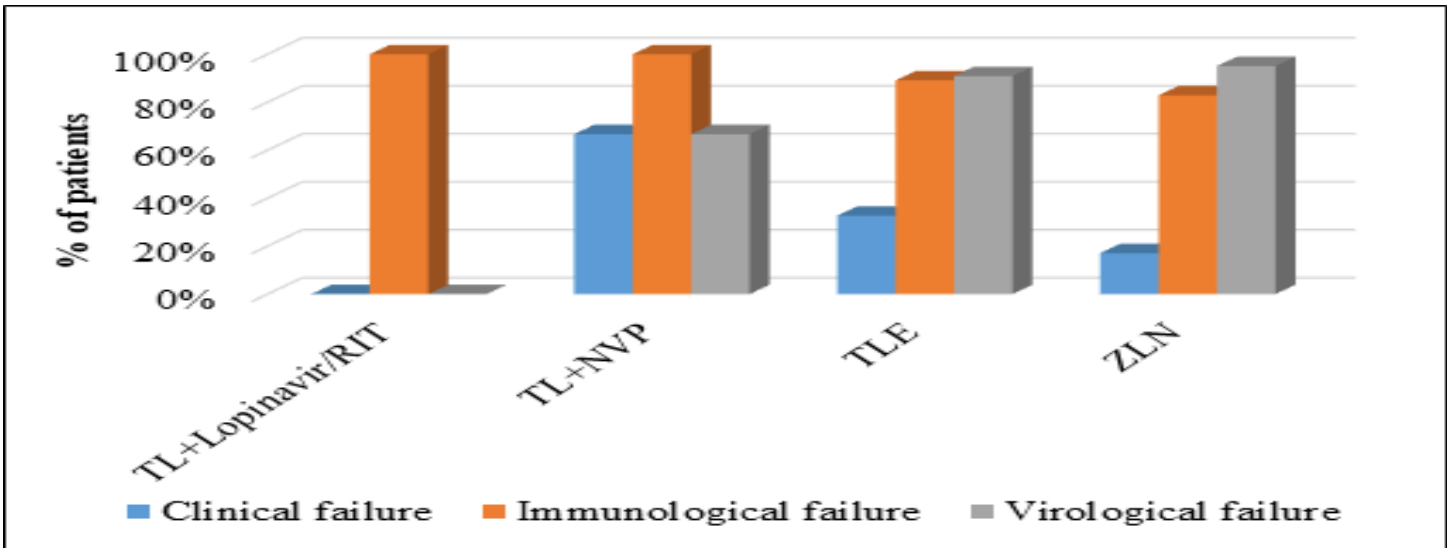
Table 6: Association of virological failure with clinical and immunological failure (N=100)

Clinical Failure	Virological Failure		Immunological Failure	Virological Failure	
	Yes	No		Yes	No
Yes	25 (92.6)	2 (7.4)	Yes	81 (93.1)	6 (6.9)
No	66 (90.4)	7 (9.6)	No	10 (76.9)	3 (23.1)
P Value = 0.735			P Value = 0.057		
Sensitivity = 27.5 %			Sensitivity = 89.1 %		
Specificity = 77.8 %			Specificity = 33.3 %		
Positive Predictive Value = 92.6%			Positive Predictive Value = 93.1%		
Negative Predictive Value = 9.6%			Negative Predictive Value = 23.1%		

Tenofovir based regime were associated with clinical, immunological and virological failure. Most of the subjects were on Tenofovir + Lamivudine + Efavirenz regime (55%) of which 50 (90.9%) subjects were

associated with virological failure. Also, of 41 subjects who were on Zidovudine + Lamivudine + Nevirapine regime, 39 (95.1%) were associated with virological failure as depicted in figure 2.

Figure 2: Association between current regime and clinical, immunological and virological failure (N=100)



Subjects with 70-80% adherence to ART shows 100% (3/3) virological failure while virological failure was present in 75% (3/1) subjects with 81-90% adherence to ART. Virological failure was 100% in subjects with 91-95% adherence to ART. 91.2% (82/90) subjects were having virological failure while remaining 8.8% (8/90) were non-virological failure. P value= 0.601 not significant.

Discussion

The outcome of ART can be monitored using different criteria's including virological, immuno logical, and clinical evaluations. Virological evaluation (HIV RNA measurement) is generally accepted as the gold standard, for monitoring of treatment outcome. Using this method, early treatment failure can be detected and a change to other more effective antiretroviral regimens is then possible. However, in resource-limited settings, like most developing countries including India, the high cost of periodic viral measurements makes it almost impossible for all people in these developing countries to access ART with effective monitoring. Therefore, two cheaper surrogate markers are routinely used to monitor the efficacy of therapy in the developing world, which are: clinical assessment and CD4+ cell count. HIV RNA measurement (Viral load) is generally reserved for use in patients with immunological and/or clinical failure. The availability of free HIV RNA viral load testing through NACO funds for all our subjects at our ART center was a major advance which made this study possible.

In the present study, majority of subjects were HIV 1, but we encountered single case of HIV 2 during our study. This reflects the worldwide distribution of HIV 1 and also emerging strains of HIV 2 worldwide. Mean age of study population was 39.95 years, ranged from 13-69 years which is comparable with the study done by Hailu et al [10] and Bishnu S et al [11]. There was not significant difference, but still male population was slightly higher as compared to females as similar to Penot P et al [12]. The first reason relates to gender differences in pharmacokinetic and pharmacodynamics profiles of ART [13]. The second relates to a worse adherence to HIV care and treatment in men, as frequently observed in Africa [14]. Most subjects were married (96%) because of social pressure to get married at young age in India,

even though P value was significant which is similar to the other studies [10, 15]. This might be due to the late diagnosis of HIV status, reluctance to use condoms and non-disclosure of HIV status to spouse and indicates the need for more comprehensive and family-oriented HIV testing. Majority were heterosexual (92%). Homosexual group were 2 with both having virological failure hence there`s a need to conduct study involving homosexual in regard to risk, incidence and prevalence of HIV and emerging strains in larger population. Duration of follow up of ART >7 years was associated with treatment failure as well as virological failure. This might be due to the fact that subjects included in study were referred from other follow up centers and maximum population shows good adherence >95% to ART. The maximum subjects were in WHO clinical stage 1 (57%) and only 19% and 7% were in WHO clinical stage 3 and 4 respectively. There were 19% subjects with WHO clinical stage 2. This is similar to the study done by Bishnu S et al [11]. There was significant reduction in CD4 count at presentation and 6 months before referral to DACEP for second line ART. Also, reduction in CD4 count was significantly associated with clinical failure. This correlates clinical and immunological failure due to ART unresponsiveness further leading to virological failure. Similar findings are reported in Patrikar S et al study [16].

We encountered cases with ADR to First line ART which includes Efavirenz induced rash (2), Tenofovir induced nephrotoxicity (4) & Zidovudine induced anemia in 6 cases which is comparable with the previous studies [17-19]. Most prevalent opportunistic infection was pulmonary and extra pulmonary tuberculosis along with oral candidiasis which was consistent with the other studies [16, 20]. Age and gender do not significantly affect the ART/treatment failure (clinical, immuno logical, and virological as defined by NACO guidelines. Virological failure was not significantly associated with clinical failure in current study (P value= 0.735). Sensitivity of predicting virological failure in subjects with clinical failure was 27.5% and specificity was 77.8% which is correlated with the study conducted by Laurent C et al [21]. Also, virological failure was not significantly associated with immunological failure. Sensitivity of predicting virological failure in subjects with immunological failure was 89.1% while specificity was 33.3% which is consistent with the previous studies [22, 23].

In present study group 10% of subjects showed non adherent to the treatment <95% while remaining 90% where adherent to the ART. This shows that even if adherence is good it can lead to virological failure. Nonadherence is one of the main reasons for failure of first line ART. In fact, it is the second strongest predictor of progression to AIDS and death, after CD4 count. Incomplete adherence to ART is common in all groups of diseased individuals. The average rate of adherence is approximately 70%. It is a well-known fact that long-term viral suppression requires a near perfect adherence to ART. The resulting virologic diminishes the chances of for long-term clinical success. Non-adherence is therefore a major modifiable factor in undermining the dramatic improvements in HIV-related health parameters seen in countries where ART is widely available. Out of 100, 55% of referred subjects were on TLE, 41% subjects were on ZLN, 3 % were on TL + NVP while 1% was on TL + Lop/rit. Tenofovir based regime were associated with clinical, immunological, and virological failure. Most of the subjects were on Tenofovir + Lamivudine + Efavirenz regime (55%) of which 50 (90.9%) subjects were associated with virological failure. Also, of 41 subjects who were on Zidovudine + Lamivudine +

Nevirapine regime, 39 (95.1%) were associated with virological failure. This result is inconsistent with the study done Ayele G et al [15]. This inconsistency might be due to the fact, distribution of strains of HIV and emerging new drug resistant strains. In our study group 32% referred subjects were switched to TL + ATA/rit, 29% were switched to ZL + ATA/rit and 18% were switched to ABC/LAM + ATA/rit as per NACO guidelines. 1 was referred to SACEP as a case of HIV 2.

Conclusion

In conclusion, subjects with low baseline CD4 show impaired response to ART despite of virological suppression leading to treatment failure. Even though only 10% subjects were poorly adherent to ART, non-adherence is most important predictor of treatment failure in low-middle socio-economic countries where resources are limited along with clinical parameters. Sensitivity and specificity of clinical/immunological failure alone in predicting virological failure was poor. This highlights the need for considerations of all parameters including viral load (gold standard) before declaring virological failure.

Limitations

Most of the subjects in current study were HIV-1 (99%) and only 1% was HIV-2; Adherence was assessed on the basis of pill count which relies upon the facts provided by patient. Being a single center study from a referral center, the data from this study might not accurately reflect data from across the nation or region. Single viral load measurement was done due to limited resources. Acquired drug resistance testing was not done, because the testing facility has not been so far available. Given the high prevalence of HIV in INDIA, a much larger cohort of patients would have to be studied to enable extrapolation of these results to the large populations in the community.

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