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Original Research Article

Clinical progression of opportunistic infections and their correlation with CD4 count in newly diagnosed HIV positive patients when attending tertiary care hospital

Vasanti C. Visawale[1]*, Shilpa S. Patil[2], Suvarna B. Landge[3]

[1]Government Medical College, Ambernath, Maharashtra, India
[2]Grant Government Medical College, Mumbai, Maharashtra, India
[3]Kashibai Navale Medical college, Pune, Maharashtra, India

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***Correspondence:**
Dr. Vasanti C. Visawale,
E-mail: dr.vasu1694@gmail.com

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ABSTRACT

Background: HIV is a great threat faced by humanity in India. Opportunistic infections (OIs) arise due to progressive decline in CD4 count. HIV related OIs are the major causes of morbidity and mortality. The most prevalent OI reported in India is tuberculosis followed by candidiasis and diarrhoeal diseases. The study aimed to evaluate spectrum OIs in newly diagnosed HIV positive patients and their relation with CD4 count.
Methods: A prospective observational study was conducted over a period of 18 months from January 2021 to June 2022 at ART centre of a tertiary care hospital. All newly diagnosed HIV positive patients were clinically evaluated and monitored for absolute CD4 count by flow cytometer.
Results: A total of 117 newly diagnosed HIV patients were enrolled, of whom 100 patients completed two follow -up. Among these, 65% were males and 35% females. The mean CD4 cell count was 289.04 cells/mm^3.OIs were observed in 57% of patients. Of these, at ART initiation,71.93% patients had OIs, while 24.56% and 3.51% developed during 1st and 2nd follow up respectively. Tuberculosis was found to be most prevalent infection among HIV patients. Of total OIs, 24.56% patients of pulmonary tuberculosis and 17.54% extrapulmonary tuberculosis, followed by Oral candidiasis (26.31%).
Conclusions: Tuberculosis was the most common OI in newly diagnosed HIV positive patients. OIs were more frequent in patients with lower CD4 counts. Early diagnosis, prompt initiation of treatment and periodic monitoring of CD4 count were key to manage HIV related OIs.

Keywords: HIV, CD4 count, Opportunistic infection, Tuberculosis

INTRODUCTION

Human Immunodeficiency virus (HIV) is a great threat faced by humanity in resource limited countries like India. Despite all efforts made to prevent the infection, it still constitutes a major burden of infectious diseases. As per UNAIDS report 2023, globally 39.9 million people living with HIV.[1] In 2023, 3 million HIV positive people were newly diagnosed, of which 68,000 were reported in India.[1,2] HIV targets CD4+T lymphocytes, which are essential for triggering and controlling the immunological response and destroys the immune system resulting in immunodeficiency condition.[3] Progressive decline in CD4 levels increases risk of developing opportunistic infections (OIs) and mortality.[4] Clinical severity and death in AIDS patient vary with the type of opportunistic infections or malignancy present.[5] Depending on the prevalence of infections detected in various regions of the country, the profile of OIs varies. The most prevalent OI reported in

India is tuberculosis (pulmonary and extrapulmonary) followed by candidiasis and diarrhoea. Also, other bacterial, viral, fungal and protozoal infections are common in PLHIV.[6] Opportunistic infections affect health and quality of life of PLHIV. OIs continue to be the predominant cause of HIV-associated morbidity and mortality mainly in low- and middle-income countries. Due to the broad availability of antiretroviral therapy (ART), the prevalence of OIs has significantly decreased over time. Depleted CD4 count results in immunopathogenesis of HIV infection and disease progression. Thus, CD4 T lymphocyte cell count is used for monitoring HIV disease progression and response to ART.[7] As CD4 count decreases, management of HIV patients with OIs becomes difficult. Hence, early diagnosis of OIs in HIV positive patients is necessary as prompt initiation of treatment can slow down the disease progression. It also improves the quality of life and helps in reduction of morbidity and mortality due to HIV related OIs. Thus, this study was aimed to evaluate opportunistic infections in newly diagnosed HIV positive patients and clinical progression of OIs in relation with CD4 count.

METHODS

A prospective observational study was conducted in State Reference Laboratory, Department of Microbiology at a tertiary care hospital, Mumbai. The study was carried out over a period of 18 months from January 2021 to June 2022. The details of the study were presented and approved by the Institutional Ethics Committee of tertiary care hospital, Mumbai. Informed consent was taken from all patients involved in the study. A total of 117 newly diagnosed HIV positive patients enrolled at ART Centre, tertiary care hospital, Mumbai were included in the study from January 2021 to June 2022. The patients were followed up at baseline (at ART initiation), 6 months and 12 months for CD4 testing. Only newly diagnosed HIV-1 positive patients, more than 18 years and consenting to the study were included. Pregnant women, HIV-2 patients and patients not consenting were excluded from the study. All newly diagnosed HIV positive patients after registration at ART centre were evaluated clinically and underwent CD4+T lymphocyte count as a baseline investigation. Clinical and immunological monitoring was done at 6 months and 12 months after ART initiation. For CD4 T lymphocyte count, blood sample of each patient was collected in ethylene diamine tetra acetic acid (EDTA) tubes. Absolute CD4+T cell count was done on whole blood with the Sysmex CyFlow Counter flow Cytometer. The statistical analysis was done using Statistical Package for the Social Sciences (SPSS) (Version 20.0.) Descriptive statistics and Wilcoxon Signed Rank Test were performed.

RESULTS

A total of 117 newly diagnosed HIV positive patients enrolled at ART center of a tertiary care hospital were screened. Each patient was evaluated and followed up for clinical progression. However, only 100 patients were finally assessed. 17 patients could not be assessed due to loss to follow up, transfer out of patients and death of patients enrolled. Of the 17 patients enrolled 7 patients transferred out, 4 patients' loss to follow up and 6 patients (5.17%) died within 6 months of initiation of antiretroviral therapy (ART). Of the dead patients 5 patients had WHO clinical stage II-IV disease. Of these 5, one patient had pharyngitis and tonsilitis, 3 had PTB and 1 had EPTB. All 6 patients had a CD4 count of < 250 cells/mm^3.

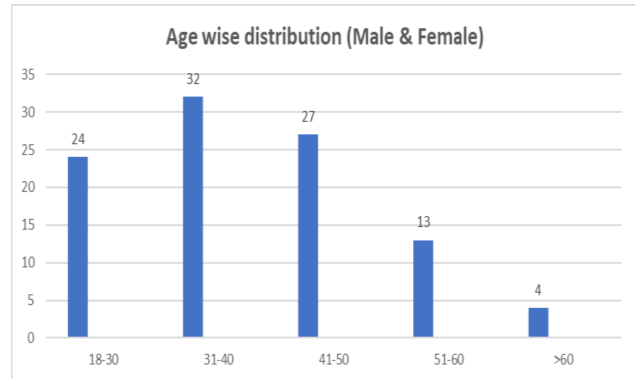

Figure 1: Age wise distribution.

The results of 100 patients who completed two follow ups over a period of 12 months is as follows. Of these 100 patients, 65 were male and 35 were female.

Thus, the male:female ratio was 1.8:1. Predominant affected age group was 31-40 years. In males most affected age group was 31-40 years and in females was 18-30 years. Mean±SD age of HIV positive patient was found to be 39.61±11.58 (Figure 1).

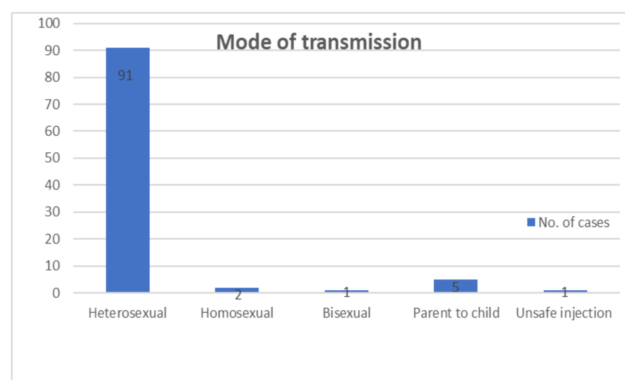

Figure 2: Mode of transmission.

Of the 100 HIV positive patients, 56 patients were married, followed by 24 patients who were unmarried. 12 patients were widowed and 8 were divorced/separated. The most common mode of transmission of HIV infection was heterosexual route in 91 patients followed by parent to child transmission in 05 patients. Clinical monitoring of the newly HIV patients was done by WHO staging. Only 49 patients were categorised in WHO stage I, whereas 51 patients belonged to WHO stage II-IV.

Table 1: WHO clinical staging of newly HIV patients.

	Total patients			P value
Stage	Baseline	1st F/U	2nd F/U	
I	49	79	97	0.0000
II	10	01	01	
III	26	07	01	
IV	15	13	01	

Table 2: Baseline CD4 count of newly HIV patients.

Baseline CD4 cells/mm3	Total
<200	44
200-349	29
350-499	9
≥500	18
Total	100

During 1st follow up 79 patients with WHO stage I and 21 patients in WHO stage II-IV. In 2nd follow up there was further clinical improvement in patients with 97 patients moving to Stage I and only 3 belongs to Stage II-IV. Thus, there was a drastic improvement in the clinical status of the patients with the initiation of anti-retroviral therapy. (Table1). There is a significant association between WHO clinical stage during baseline and follow-ups (P value <0.05). Baseline CD4 count was done at ART initiation, 44 patients had a count < 200, followed by 29 patients who had between 200-349 and 9 patients had between 350-499. Only 18 patients had a CD4 count more than 500 cells/mm^3. Mean CD4 cell count of study group was 289.04 cells/mm^3. Overall prevalence of OIs was found to be 57%. Tuberculosis was found to be most prevalent infection among HIV patients (42%). Of this 24.56% patients of pulmonary tuberculosis and 17.54% extrapulmonary tuberculosis. Followed by oral candidiasis (26.31%), Herpes zoster virus and Bacterial Pneumonia (7.01%) each, diarrhoea and Herpes simplex virus (5.26%) each. Of 57 patients, 41(71.93%) patients had OIs at ART initiation, followed by 14(24.56%) and two (3.51%) patients presented with OIs during 1st and 2nd follow up respectively. Of 41 patients At ART initiation, 12 patients had PTB followed by 11 patients with oral candidiasis. During 2nd follow-up, only two patients had oral candidiasis as opportunistic infection. Thus, it was observed that the number of patients presenting with opportunistic infections during 1st and 2nd follow-up decreased after ART initiation (Table 2).

Table 3: Distribution of opportunistic infections in newly HIV patients.

Sr. No	Opportunistic infections	Baseline	First follow up	Second follow up	Total	%
1	Pulmonary tuberculosis (PTB)	12	2	0	14	24.56
2	Extra-pulmonary tuberculosis (EPTB)	7	3	0	10	17.54
3	PTB and PCP	0	1	0	1	1.75
4	EPTB and diarrhoea	1	0	0	1	1.75
5	Oral candidiasis	11	2	2	15	26.31
6	Oral candidiasis and HSV	0	1	0	1	1.75
7	Oral candidiasis and bacterial pneumonia	0	1	0	1	1.75
8	Diarrhoea	3	0	0	3	5.26
9	Herpes simplex virus (HSV)	2	1	0	3	5.26
10	Herpes zoster virus (HZV)	2	2	0	4	7.01
11	Bacterial pneumonia	3	1	0	4	7.01
	Total	41(71.93%)	14(24.56%)	2(3.51%)	57	100

Table 4: Distribution of patients with opportunistic infections and CD4 count.

CD4 cells/mm3	Baseline	First follow up	Second follow up	P value
<200	30	5	1	0.00
200-349	9	7	1	
350-499	1	2	0	
≥500	1	0	0	
Total	41	14	2	

Of 41 patients who had OIs at ART initiation (baseline), 30 patients had CD4 count <200 and 11 patients had CD4 count ≥ 200. In 1st follow up, of 14 patients who had OIs, 5 patients had CD4 count <200 and 9 patients showed CD4 count ≥ 200. In 2nd follow up, of 2 patients who presented with OIs, 1 patient each had count <200 and between 201-349. Decreased CD4 count at ART initiation has higher chances of developing OIs. There is significant association

between CD4 count during baseline and follow-ups (P value<0.05)

DISCUSSION

In the present study newly diagnosed HIV positive patients were evaluated by clinical and immunological monitoring at baseline at the time of ART registration and followed further at 6 months and 12 months of initiation of ART.

A total of 117 newly diagnosed HIV positive patients were enrolled in the study initially. Of these, 17 patients could not to be followed up further. The reason was that 7 patients were transferred out (5.98%), 4 patients were lost to follow up (3.41%) and 6 patients died (5.17%). A study conducted by H Gautam et al (2008) at Maulana Azad Medical college, New Delhi reported loss to follow up in 21% and death in 19% cases in their study.[8] 7 patients were transferred out to ART centre near their place of residence.[9] since there has been a massive scale up and decentralization of ART services. Loss to follow up was seen in 4 patients due to various social determinants like low education, homelessness, poverty, drug toxicity, cultural factors, beliefs about the treatment and mental illness etc.[9]

Death was reported in 6 patients (5.17%) in our study among the 17 patients. Of these 6 patients, WHO Clinical staging showed that 1 (16.66%) patient was asymptomatic (stage I), 1 (16.66%) patient was in stage II with the pharyngitis and tonsilitis, 3 (50%) patients were in stage III with PTB and 1 (16.66%) patient suffered from EPTB and was in stage IV of HIV disease. All these patients had a CD4 count between 25 to 250 cells/mm^3. All these patients died within 6 months of initiation of ART. A study by S. Konrad et al (2013), Canada has reported death in 6.7% of their study populations.[10] A study conducted by H Gautam et al (2008) showed that 21% belonged to WHO stage IV, 51% to stage III and 28% to stage II. All patients who expired in their study had a CD4 count between 7-69 cells/μL.[8] The reason of death in these 6 patients was severe immunosuppression and start of ART with clinical stage IV.

Thus, only 100 patients were studied further over 12 months and discussion is as follows. In the present study 65% patients were male and 35% patients were female. The male:female ratio was 1.8:1. Similar findings have been documented in a study by Monika Advani et al (2020) from JLN Medical College, Ajmer. This study had 55.30% males and 44.67% females.[11] Another study conducted at MAMC, New Delhi by Sanjim Chadha et al had male: female ratio of 2.1:1.[12] These findings are in concordance with our study. That, infection is common in males.

In the present study, Predominant affected age group was 31-40 years. In males most affected age group was 31-40 years and in females was 18-30 years**.** Mean±SD age oHIV positive patient was found to be 39.61±11.58. Study by Trinh Duong et al in Thailand documented 30-39 as predominant age group affected with 47% in the said group.[13] Sanjim Chadha et al MAMC, New Delhi have mentioned 26-30 years as the predominant age group in males and females.[12] All the above studies are in concordance regarding the predominant age group affected. According to a study conducted by Simone E Langford et al Monash University, Melbourne, Australia there is a clear relationship between increasing risk with increasing age. Older age is accounted with lower CD4 count and faster progression which may explain the relationship between age and decrease progression. However, age disparities seem to diminish with ART treatment.[14]

In the present study married people were most commonly affected (56%). There were 12 widows and 8 separated /divorced patients. An important finding was that 24% patients in our study were unmarried, thus reiterating the high risk in these cases. Paotinlal Haokip (2013) RIMS, Imphal in their study have documented 93.9% cases to be married.[15] Another study by Abere Kassie, Debre Berhan University (2022) has reported married patients in 48.14%, never married 20.50%, Divorce 13.98% and separated and widowed 17.39%.[16] Thus, the above two studies are in concordance with our study.

The most common mode of transmission in our study was heterosexual in 91% cases. Sanjim Chadha et al (2013) MAMC, New Delhi, have documented that the most common mode of acquiring infection was heterosexual contact (85.38%) followed by intravenous drug abuse (9.2%).[12] Study by Paotinlal Haokip et al (2018), Manipur documented heterosexual route (75.7%) and injecting drug use in (19.5%).[15] On clinical monitoring it was observed that 49% patients were categorized a stage I, 10% as stage II, 26% stage III and 15% as stage IV. On subsequent follow up, it was found that there was drastic improvement in the patients from baseline to the 2nd follow up. This data was found to be statistically significant (p value <0.05). A study conducted by H Gautam et al (2008) MAMC, New Delhi showed that 21% belonged to WHO stage IV, 51% to stage III and 28% to stage II. On initiation of ART with > 95% adherence, 80.8% showed clinical improvement.[8]

In the present study, immunological monitoring was done by estimation of CD4 count before initiation of ART. It was observed that at baseline testing 44% patients had a CD4 count < 200 cells/mm^3 and 18 % had a CD4 count > 500 cells/mm^3. Thus, maximum number of patients enrolled in the study had a CD4 count of < 200 cells/mm^3 which is suggestive of advanced HIV disease.

In a study by Monika Advani et al (2020), JLN Medical College Ajmer, CD4 count of >500 was seen in 19 subjects, while CD4 count of 0-200 cells/mm^3 was seen in 102 subjects.[11] Another study by Claris Shoko and Delson Chikovu, (2019), University of Venda had approximately 70% of the study population with a baseline CD4 count below 200 cells/mm^3.[17] Thus, it is observed from the above study that most patients seek health only with clinical and

immunological deterioration when they reached advanced HIV disease.

In the present study we tried to analyse the various OIs in our study group throughout the follow up. Overall prevalence of OIs in present study was found to be 57%. The finding is in concordance with the study conducted by Kallol S et al (2011) in Kolkata, India which documented 53.4%.[18] whereas the prevalence is far lower than the study conducted in Bahir Dar, Ethiopia which reported 88.9% prevalence.[19] Tuberculosis (pulmonary and extrapulmonary) emerged to be most common OIs among HIV positive patients followed by oral candidiasis.

Tuberculosis was found to be most prevalent infection among HIV patients (42%). Of this 24.56% patients of pulmonary tuberculosis and 17.54% extrapulmonary tuberculosis. Followed by oral candidiasis (26.31%), Herpes zoster virus and Bacterial Pneumonia (7.01%) each, diarrhoea and Herpes simplex virus (5.26%) each.

It was observed that a total of 57 patients presented with OIs, of which 41 patients (71.93%) had OIs during baseline on initiation of ART. There was reduction in the OIs to 14 (24.56%) during 1st follow up and only 2 patients (3.51%) had OIs during 2nd follow up. Of these 57 patients with OIs the most common OIs documented at ART initiation were: 12 patients had PTB followed by Oral candidiasis in 11 patients, 7 patients had EPTB whereas diarrhoea was seen in 3 patients. Similar study carried out by H. Gautam et al (2008) MAMC, New Delhi, have reported 53 patients with OIs in their study which reduced to 4 patients with OIs on initiation of HAART. The most common OI was PTB followed by diarrhoea, oral candidiasis and herpes zoster.[8]

A study conducted by Praveen Shahapur and Rajendra Bedri (2014), Shri BM Patil Med College Bijapur, Karnataka have also observed PTB as the most common OI followed by candidiasis, cryptosporidial diarrhoea, herpes zoster, cryptococcal meningitis and P. jirovecii pneumonia.[20] Both the above studies are in concordance with our findings. Strict adherence to ART, improved hygienic practices, regular examination and appropriate antimicrobial prophylaxis can reduce the substantial morbidity and mortality caused by OIs in patients with HIV.

In the present study a correlation of CD4 count was done with OIs. Of the 41 patients with OIs at ART initiation, 30 patients had a baseline CD4 cell count <200 cells/mm^3, 9 had a baseline CD4 cell count between 200-349 cells/mm^3 and 1 each had a CD4 count between 350-499 cells/mm^3 and >500 cells/mm^3. 30 patients with OIs and a baseline CD4 cells count of <200 cells/mm^3, when followed up after initiation of ART there was a steep fall in the OIs from 30 to 5 during 1st follow up which further dropped to 1 during the 2nd follow up. This was associated with an improvement in CD4 count. This finding was found to be statistically significant (P<0.000). The most common OI at baseline was PTB and oral candidiasis followed by EPTB and chronic diarrhoea. During 1st and 2nd follow up there was no cases of PTB and the most common OI was EPTB and oral candidiasis. Similar finding was seen in patients with higher CD4 counts of 200-349 at baseline which showed a drop in OIs at follow up and further improvement in CD4 count. (P-0.001).

Patients with baseline CD4 between 350-499 cells /mm^3 and >500 cells/mm3, no OIs were documented in second follow up. Systematic reviews done in various low to middle income countries by Low A et al (2016), it was seen that ARTs have a huge impact and led to greatest reduction in the incidence of all OIs during the first 12 months of ART with an improvement in the CD4 count.[21] Thus, the finding shows that higher the CD4 count during ART initiation, less are the chances of developing OIs and progression of OIs. Thus, CD4 is a strong predictor of OIs and disease progression and survival.

Limitations

Limitations of the study were HIV positive patients who diagnosed but not attended ART centre for follow ups, conducted the study only on HIV-1 patients, paediatric patients, pregnant women and adolescents below 18 years were not included in the study.

CONCLUSION

Opportunistic infections (OIs) remain a major cause of morbidity and mortality in HIV positive patients. Their incidence is strongly influenced by level of immunosuppression and presence of specific pathogens. Early diagnosis and treatment, along with the timely initiation of antiretroviral therapy (ART), play a critical role in reducing plasma viral load, restoring immune function and preventing disease progression. Regular monitoring of CD4 cell counts is essential for assessing immune status, predicting the risk of OIs and guiding clinical decisions especially in patients with advanced HIV. Improved adherence to ART enhances treatment success and lowers the chances of drug resistance. Overall, integrated management through early intervention, consistent monitoring and patient centered care significantly improves the quality of life and survival HIV-infected individuals.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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