International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 16, Issue 4, 2024

Original Article

# TUBERCULOSIS AND ITS ASSOCIATION WITH CD4+T CELL COUNT AND VIRAL LOAD AMONG HIV POSITIVE PATIENTS IN A TERTIARY CARE HOSPITAL

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***Received: 16 Apr 2024, Revised and Accepted: 07 Jun 2024***

**ABSTRACT**

**Objective:** To assess the risk of development of tuberculosis among HIV patients and to know the correlation between CD4+T cell count, viral load and tuberculosis in follow-up cases of HIV.

**Methods:** The present study was a prospective study conducted from April 2022 to March 2023 in 4551 patients. Samples of 238 patients newly diagnosed with HIV attending Integrated Counselling and Testing Centre (ICTC), Visakhapatnam, Andhra Pradesh, India and registered for Antiretroviral Therapy (ART) were taken. 5 ml of blood sample was collected aseptically and tested for HIV. The 238 HIV-positive samples were tested for CD4 counts at presentation and after 6 mo using flow cytometry (Sysmex Partec CyFlow Flow cytometer). They were further subjected to real-time RT-PCR to detect viral load at 6 mo follow-up.

**Results:** Out of 238 HIV-positive cases, predominant gender being males with 140 (58.82%) and the predominant age group was 31-40 y. At 6 mo follow-up, 19 patients (8%) were diagnosed as having TB. The mean CD4 counts at baseline and after 6 mo of antiretroviral therapy (ART)) was 296±229 and 436±271 cells/mm$^3$ (p value of<0.001) for entire study group. The mean baseline CD4 count in patients who were not diagnosed with TB at the time of follow-up and those who were diagnosed as having developed TB was 307±232 cells/mm$^3$ and 167±135 cells/mm$^3$ respectively. At six mo follow-up, 32(14.6%) HIV patients who did not develop TB and 8(42.1%) patients who developed TB still had their CD4 counts<200 cells/mm$^3$. This was statistically significant with a p value=0.019. Significant difference was not found between the two subgroups as the HIV-only group had 192(87.7%) patients and 16(84.2%) patients from newly diagnosed TB patients had their viral loads below detection levels.

**Conclusion: l**ow CD4 counts at the baseline was a high-risk factor for the development of tuberculosis in HIV patients. The viral load values at 6-month follow-up did not prove to be significantly linked to the development of tuberculosis.

**Keywords:** Antiretroviral therapy, Mycobacterium tuberculosis, People living with HIV, Immunosuppression, Protease inhibitors


DOI: https://dx.doi.org/10.22159/ijcpr.2024v16i4.5042 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

## INTRODUCTION

Tuberculosis caused by *M. tuberculosis* complex, primarily *M. tuberculosis* (Koch's bacillus) and rarely *Mycobacterium bovis* or *Mycobacterium africanum* [1] remains the world's second leading cause of death from a single infectious agent. In 2022, the estimated number of deaths from TB among People living with HIV(PLWH) were 1,67,000 [2]. There is a synergism in TB-HIV co-infection: HIV infection makes a person more susceptible to TB, and TB also expedites the transition from HIV infection to AIDS [3].

The incidence of TB in PLWH has dropped significantly as a result of widespread anti-retroviral therapy (ART) [4].

Interferon-gamma (IFN-γ), interleukin-2, tumour necrosis factor-alpha (TNFα), and macrophage colony-stimulating factor produced by CD4+T-lymphocytes and Tγδ-lymphocytes activate macrophages and cytotoxic cells to inhibit the intracellular growth of the bacilli within the macrophages that enter through the respiratory tract [1].

These proinflammatory cytokines produced by tuberculous granulomas (in particular TNFα) might accelerate the course towards severe immunosuppression by increasing HIV (human immunodeficiency virus) viraemia [1].

Pulmonary TB cases (70-93%) usually present with cough and expectoration along with rare symptoms like haemoptysis, thoracic pain and dyspnoea. Atypical features like diffuse lower lobe involvement may be frequently seen. Cavitary lesions are usually encountered rarely in patients with a CD4 T-lymphocyte count<200/mm$^3$ [5].

Extrapulmonary TB can affect any organ in the body and commonly involves pleura, lymph node, central nervous system, abdominal organs, pericardium, and bone and often presents as disseminated TB [6]. Advanced immunosuppression may further increase the risk of extrapulmonary TB.

In severely immunocompromised HIV-infected patients, pulmonary basal involvement, tuberculous pneumonia, hilar or mediastinal lymphadenopathies and miliary TB are common. Unilateral or bilateral Pleural effusions are found in 5% of cases [1].

New World Health Organisation (WHO), as well as National Tuberculosis Elimination Program (NTEP) guidelines of India, recommend the use of rapid molecular diagnostic test/NAAT (CBNAAT or Truenat) in various samples, including cerebrospinal fluid, lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid, urine, and blood as the preferred diagnostic technique for TB testing in PLWH [7]. Adenosine deaminase, an enzyme involved in purine metabolism (in pleural and other fluids) and new diagnostic tests like Xpert Ultra and lateral flow urine lipoarabinomannan assay (LF-LAM) can also be used for diagnosis [6].

During HIV infection, drastic decrease in IFN-γ production and CD4+T-lymphocytes leads to a markedly increased risk of reactivation or reinfection by *M. tuberculosis* [1] due to lymphocytopenia and downregulation of these immune cells. People living with HIV(PLWH) are 21 times more likely to develop TB than their seronegative counterparts [8].

HIV Ribonuceic acid(RNA) (viral load) and CD4 T lymphocyte (CD4) cell count are the two important markers that are used to evaluate the treatment responses and disease progression of HIV [9].

Drug interactions between drugs used for treatment of TB and new anti-retroviral drugs (Highly Active Antiretroviral Therapy) has complicated the therapeutic management of these diseases.

Rifampicin induces Cytochrome P450(CYP) (membrane haemoproteins), found mostly in the liver and intestinal tissues and thereby lowers the concentrations of PIs (Protease inhibitors) (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir,

lopinavir/ritonavir and atazanavir) and NNRTIs (Non-nucleoside reverse transcriptase inhibitors) (nevirapine, efavirenz and delavirdine) to sub-therapeutic levels.

It also induces P-Glycoprotein, a drug efflux pump system coded by the multidrug resistance 1 gene (MDR1), which confers resistance to chemotherapy or reduces the efficacy of PIs, by diminishing the intracellular disposability of drugs.

Low plasma levels of these anti-retroviral drugs may be associated with incomplete viral suppression and the emergence of drug resistance [1]. Concomitant administration of rifampicin with these drugs often requires modification of the anti-retroviral drug dosage.

When HIV-infected patients are treated effectively for their TB and have commenced HAART (mean 15±11 d afterwards), a paradoxical worsening of signs and symptoms of TB may occur. These paradoxical reaction consist of a hectic fever, the occurrence or enlargement of lymphadenopathies, worsening of chest infiltrates, and an increase of pre-existing TB lesions (cutaneous and peritoneal) [10]. These reactions are self-limited, lasting for 10-40 d and are related to immune restoration and not to a failure to control infection. However, severe reactions may require a short course of glucocorticoids in order to attenuate the granulomatous reaction [10].

The significant number of PLWH who are undiagnosed, diagnosed late, or not diagnosed until they present to healthcare providers with other opportunistic illnesses like TB complicates efforts to prevent HIV-TB. Due to medication side effects, drug-drug interactions, and typically a more severe presentation of the disease, managing TB in PLWH is also more clinically challenging; for this reason, prevention of TB in people living with HIV is crucial [11].

**Aim of the study**

To assess the risk of development of tuberculosis among HIV patients and to know the correlation between CD4+T cell count, viral load and tuberculosis in follow-up cases of HIV.

**MATERIALS AND METHODS**

The present study was a prospective study conducted from April 2022 to March 2023 in 4551 patients. Samples of 238 patients newly diagnosed with HIV of all age groups and both sexes attending Integrated Counselling and Testing Centre (ICTC), Visakhapatnam, Andhra Pradesh, India and registered for ART according to 2021 NACO guidelines were taken. 5 ml of blood sample aseptically was collected from patients after taking informed consent and counselling in ICTC. The positive samples were tested for CD4 counts at presentation and after 6 mo using flow cytometry (Sysmex Partec CyFlow Flow cytometer) [13]. They were further subjected to real-time RT-PCR to detect viral load at 6-month follow-up [13].

**RESULTS**

Out of the total 4551 samples tested, 238 were found to be HIV positive (table 1).

**Table 1: No. of patients diagnosed of HIV**

| Total no of samples tested | Total no of hiv positive patients |
|---|---|
| 4551 | 238 |

Out of 238 HIV positive cases, males were 140(58.82%), females were 94(39.49%) and 4(1.68%) were transgenders (table 2).

**Table 2: Age and gender-wise distribution of HIV-positive patients**

| Age in years | Gender | | | Total |
|---|---|---|---|---|
| | Male | Female | Transgender | |
| 0-10 | 2 | 0 | 0 | 2(0.84%) |
| 11-20 | 1 | 2 | 0 | 3(1.26%) |
| 21-30 | 39 | 28 | 3 | 70(29.41%) |
| 31-40 | 43 | 34 | 0 | 77(33.35%) |
| 41-50 | 37 | 21 | 1 | 59(24.78%) |
| 51-60 | 15 | 7 | 0 | 22(9.24%) |
| >61 | 3 | 2 | 0 | 5(2.10%) |
| Total | 140 | 94 | 4 | 238 |

Age wise distribution of the HIV-positive patients is depicted in table 2. The predominant age group was found to be 31-40 y in both males 43(30.71%) and females 34 (36.17%). At 6 mo follow-up, 19 patients (8%) out of the 238 HIV-positive patients were diagnosed as having TB (table 3).

**Table 3: Number of HIV-positive patients who were diagnosed of TB at follow up**

| Total number of hiv positives | Newly diagnosed tb cases at 6-month followup |
|---|---|
| 238 | 19(8%) |

The mean CD4 count of the entire study population at baseline and after 6 mo of ART, is 296±229 and 436±271 cells/$mm^{3,}$ respectively. This improvement was statistically significant with a p-value 0.01. There was a significant improvement in mean CD4 counts in both the groups of patients, who were not diagnosed with TB at the time of follow-up (307±232 cells/$mm^3$at baseline to 449±274 cells/$mm^3$at follow-up), and those who were diagnosed as having developed TB (167±135 cells/$mm^3$at baseline and 292±186 cells/$mm^3$ at follow up) (table 4).

Table 5 depicts the distribution of CD4 levels of the two groups (n=219 and n=19) at baseline and after 6 mo. Significant improvement was observed in the HIV-without-TB group as a patient count of 84(38.4%) improved to 32(14.6%) in the<200 cells/$mm^3$ range within six months when compared to HIV-with-TB where 8(42.1%) patients still remained<200 cells/$mm^3$ levels after 6 mo. This was statistically significant with p value=0.019.

**Table 4: Comparison of mean CD4 counts of the HIV-positive patients (p<0.05)**

| Time of testing | Mean cd4 counts of all hiv positive patients (n=238) | mean cd4 counts of hiv patients who did not develop tb (n=219) | mean cd4 counts of hiv patients who developed tb (n=19) |
|---|---|---|---|
| At baseline | 296±229 cells/$mm^3$ | 307±232 cells/$mm^3$ | 167±135 cells/$mm^3$ |
| Post 6 mo follow up | 436±271 cells/$mm^3$ | 449±274 cells/$mm^3$ | 292±186 cells/$mm^3$ |

**Table 5: Distribution of CD4 levels of the HIV-positive patients at baseline and after 6 month follow-up (p<0.05)**

| Range of cd4 count (cells/mm³) | At baseline | | At 6 mo follow up | |
|---|---|---|---|---|
| | Patients who did not develop tb (n=219) | Patients who developed tb (n=19) | Patients who did not develop tb (n=219) | Patients who developed tb (n=19) |
| <200 | 84(38.4%) | 11(57.9%) | 32(14.6%) | 8(42.1%) |
| 200-349 | 67(30.6%) | 6(31.6%) | 56(25.6%) | 4(21.1%) |
| 350-499 | 28(12.8%) | 2(10.5%) | 55(25.1%) | 4(21.1%) |
| ≥500 | 40(18.3%) | 0 | 76(34.7%) | 3(15.8%) |

Distribution of viral load of study population at 6 mo follow-up is shown in table 6.

**Table 6: Distribution of viral load of HIV-positive patients at 6 mo follow-up (p>0.05)**

| Viral load (copies/ml) At 6 mo follow up | Number of HIV patients who did not develop TB (n=219) | Number of HIV patients diagnosed with TB at follow-up (n=19) |
|---|---|---|
| Below Detection levels | 192 | 16 |
| <250 | 9 | 1 |
| 251-500 | 4 | 0 |
| 501-1000 | 2 | 0 |
| >1000 | 12 | 2 |

Significant difference was not found between the two subgroups as the HIV-only group had 192(87.7%) patients and 16(84.2%) patients from newly diagnosed TB patients had their viral load below detection levels.

## DISCUSSION

In spite of collaborative TB and HIV activities, TB remains the leading cause of death in people living with HIV [6]. lack of socioeconomic development [14], such as poor living conditions characterized by overcrowding and poor ventilation create the perfect environments for TB transmission. Poor access to health care exacerbates the epidemic further.

TB may in turn exacerbate HIV infection by increasing HIV viral load in lungs, blood, and cerebrospinal fluid in people with TB due to increased viral replication at the sites of granulomatous inflammation with abundant activated T cells and upregulation of HIV transcription by pro-inflammatory cytokines produced through the host immune response against TB [6].

In the present study, the predominant gender in HIV infected was found to be males (58.82%), especially in middle-aged individuals (30.71%), which is likely to be due to high prevalence of individual risk factors (e. g., unprotected sex, intravenous drug abuse, smoking, undernutrition, and alcohol use).

The CD4 count, the most important laboratory indicator of immune status in HIV-infected patients, a strong predictor of HIV disease progression and subsequent survival rate [15] is usually decreased in TB infection in PLWH.[8]

The mean CD4 count at baseline and after 6 mo of ART, is 296±229 cells/mm³ and 436±271 cells/mm³ cells/mm³, which indicates that the CD4 levels have markedly improved post-ART in HIV infected.

The viral load is the most important indicator of response to ART and should be measured in all patients infected with HIV. In 208(87.39%) patients post 6 mo of ART, the HIV RNA viral load levels were found to be undetectable by RTPCR, which indicates good response to HAART.

For the HIV-positive cases developing TB, male gender showed slight predominance with 10 out of 19 cases being males, which is in correlation with previous studies [4, 16, 17]. Amongst the HIV-positive patients who developed TB, 11(57.9%) had baseline CD4 counts of<200 cells/mm³. The results of the current study further showed that among the PLWH, a CD4 count of fewer than 200 cells/mm3 was significantly linked to TB infection. This result is consistent with prior studies [7, 18-20].

The mean increase between baseline and post-ART CD4 counts in present study is in correlation with other studies (table 7) [21-24].

**Table 7: Mean increase in CD4 counts in various studies**

| Study | Year | Size of study population | Mean increase between baseline and post-art CD4 count |
|---|---|---|---|
| Present study | 2022 | 238 | 140 cells/mm³ |
| Chakraborty *et al.*, | 2012 | 15136 | 104 cells/mm³ |
| Mrudula *et al.*, | 2010 | 57 | 139 cells/mm³ |
| Goutam *et al.*, | 2006 | 43 | 152 cells/mm³ |

## CONCLUSION

WHO recommends TB screening based on four symptoms-cough, fever, night sweats, and weight loss. However, people living with HIV on ART are more likely to be asymptomatic, and only around 50% have one of the above four symptoms. So all patients with HIV, especially those with clinical risk factors for HIV-TB like low CD4 count and high viral load, should be screened irrespective of the symptoms for TB.

The patients with low CD4 counts at the baseline should be monitored closely for the development of tuberculosis as we could find a significant relationship between the two.

The viral load values at 6 mo follow-up did not prove to be significantly linked to the development of tuberculosis.

Limitations of the study: The baseline viral loads of the patients were not performed as they were primarily done at 6 mo(National Guidelines for HIV Care and Treatment 2021) follow-up in the ICTC centre.

## ACKNOWLEDGEMENT

We gratefully acknowledge the support rendered by the staff of ICTC, Department of Microbiology, Andhra Medical College, Visakhapatnam.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

First author of the study Nigel Jose, contributed literature search, collected the data, analyzed the data and wrote the first draft of the manuscript. The second author Aruna Bula, contributed conceptual design, data analysis, statistical analysis, literature survey and corrected the manuscript. The third and fourth authors Ratna

Kumari Poosapati and Puvvula Kamala contributed in drafting the manuscript.

**CONFLICTS OF INTERESTS**

Declared none

**REFERENCES**

1. Aaron L, Saadoun D, Calatroni I, Launay O, Memain N, Vincent V. Tuberculosis in HIV-infected patients: a comprehensive review. Clin Microbiol Infect. 2004;10(5):388-98. doi: 10.1111/j.1469-0691.2004.00758.x, PMID 15113314.
2. Global tuberculosis report; 2023. p. 2.
3. Whalen C, Horsburgh CR, Hom D, lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. Am J Respir Crit Care Med. 1995 Jan;151(1):129-35. doi: 10.1164/ajrccm.151.1.7812542, PMID 7812542.
4. Gupte AN, Kadam D, Sangle S, Rewari BB, Salvi S, Chavan A. Incidence of tuberculosis in HIV-infected adults on first- and second-line antiretroviral therapy in India. BMC Infect Dis. 2019 Oct 29;19(1):914. doi: 10.1186/s12879-019-4569-z, PMID 31664933.
5. Narita M, Ashkin D, Hollender ES, Pitchenik AE. Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS. Am J Respir Crit Care Med. 1998;158(1):157-61. doi: 10.1164/ajrccm.158.1.9712001, PMID 9655723.
6. Hamada Y, Getahun H, Tadesse BT, Ford N. HIV-associated tuberculosis. Int J STD AIDS. 2021;32(9):780-90. doi: 10.1177/0956462421992257, PMID 33612015.
7. Awadalla H, El-Samani F, Soghaier MA, Makki M. Risk factors associated with the development of tuberculosis among HIV-infected patients in Khartoum in 2010. AIMS Public Health. 2015 Dec 2;2(4):784-92. doi: 10.3934/publichealth.2015.4.784, PMID 29546135.
8. Geremew D, Melku M, Endalamaw A, Woldu B, Fasil A, Negash M. Tuberculosis and its association with CD4$^+$T cell count among adult HIV positive patients in Ethiopian settings: a systematic review and meta-analysis. BMC Infect Dis. 2020 May 7;20(1):325. doi: 10.1186/s12879-020-05040-4, PMID 32380957.
9. Cassenote AJ, Grangeiro A, Escuder MM, Abe JM, Segurado AA. Validation of CD4$^+$T-cell and viral load data from the HIV-brazil cohort study using secondary system data. BMC Infect Dis. 2018;18(1):617. doi: 10.1186/s12879-018-3536-4, PMID 30514215.
10. Aggarwal AN, Agarwal R, Sehgal IS, Dhooria S. Adenosine deaminase for diagnosis of tuberculous pleural effusion: a systematic review and meta-analysis. Plos One. 2019;14(3):e0213728. doi: 10.1371/journal.pone.0213728, PMID 30913213.
11. Winter JR, Adamu AL, Gupta RK, Stagg HR, Delpech V, Abubakar I. Tuberculosis infection and disease in people living with HIV in countries with low tuberculosis incidence. Int J Tuberc Lung Dis. 2018 Jul 1;22(7):713-22. doi: 10.5588/ijtld.17.0672, PMID 29914596.
12. National guidelines for HIV care and Treatment; 2021.
13. Jin CZ, Zhao Y, Zhang FJ, Yao HP, Wu LJ, Zhao HX. Different plasma levels of interleukins and chemokines: comparison between children and adults with AIDS in China. Chin Med J (Engl). 2009 Mar 5;122(5):530-5. PMID 19323903.
14. Hermans S, Horsburgh CR Jr, Wood R. A century of tuberculosis epidemiology in the northern and Southern Hemisphere: the differential impact of control interventions. Plos One. 2015 Aug 19;10(8):e0135179. doi: 10.1371/journal.pone.0135179, PMID 26288079.
15. Perlman DC, El-Sadr WM, Nelson ET, Matts JP, Telzak EE, Salomon N. Variation of chest radiographic patterns in pulmonary tuberculosis by degree of human immunodeficiency virus-related immunosuppression. The Terry Beirn Community programs for clinical research on AIDS (CPCRA). The AIDS Clinical Trials Group (ACTG). Clin Infect Dis. 1997;25(2):242-6. doi: 10.1086/514546, PMID 9332519.
16. Wondmeneh TG, Mekonnen AT. The incidence rate of tuberculosis and its associated factors among HIV-positive persons in sub-Saharan Africa: a systematic review and meta-analysis. BMC Infect Dis. 2023 Sep 18;23(1):613. doi: 10.1186/s12879-023-08533-0, PMID 37723415.
17. Tchakounte Youngui B, Coffie P, Messou E, Poda A, Fortes Deguenonvo l, Hawerlander D, Minga A, Balestre E, Dabis F, Marcy O. Incidence of tuberculosis during the first year of antiretroviral treatment in West African HIV-infected adults. Open Forum Infect Dis. 2020 Jun 2;7(6).
18. Darraj MA, Abdulhaq AA, Yassin A, Mubarki S, Shalaby HM, Keynan Y. Tuberculosis among people living with HIV/AIDS in Jazan Region, Southwestern Saudi Arabia. J Infect Public Health. 2021;14(11):1571-7. doi: 10.1016/j.jiph.2021.09.009, PMID 34656963.
19. Iroezindu MO, Ofondu EO, Mbata GC, van Wyk B, Hausler HP, Dh A. Factors associated with prevalent tuberculosis among patients receiving highly active antiretroviral therapy in a Nigerian tertiary hospital. Ann Med Health Sci Res. 2016 Mar-Apr;6(2):120-8. doi: 10.4103/2141-9248.181837, PMID 27213096.
20. Shivakoti R, Sharma D, Mamoon G, Pham K. Association of HIV infection with extrapulmonary tuberculosis: a systematic review. Infection. 2017 Feb;45(1):11-21. doi: 10.1007/s15010-016-0960-5, PMID 27830524.
21. Mrudula ND, Suwarna UP, Khadse R, Minal P, Shubhangi DK. Statistical analysis and evaluation of CD4 count after 6 mo on ART. Indian J Community Med. 2012 Oct;37(4):266-7. doi: 10.4103/0970-0218.103480, PMID 23293446.
22. Gautam H, Bhalla P, Saini S, Dewan R. Correlation between baseline CD4 +T-Lymphocyte count and plasma viral load in AIDS patients and their early clinical and immunological response to HAART: a preliminary study. Indian J Med Microbiol. 2008;26(3):256-8. doi: 10.4103/0255-0857.42039, PMID 18695327.
23. Chakraborty H, Iyer M, Duffus WA, Samantapudi AV, Albrecht H, Weissman S. Disparities in viral load and CD4 count trends among HIV-infected adults in South Carolina. AIDS Patient Care STDs. 2015;29(1):26-32. doi: 10.1089/apc.2014.0158, PMID 25458918.
24. Liu E, Makubi A, Drain P, Spiegelman D, Sando D, Li N. Tuberculosis incidence rate and risk factors among HIV-infected adults with access to antiretroviral therapy. AIDS. 2015 Jul 17;29(11):1391-9. doi: 10.1097/QAD.0000000000000705, PMID 26091295.