original article

CD4+T LYMPHOCYTE RESPONSE IN HIV-TB SYNDEMIC

W. Shashi Singh, Th. Nabakumar Singh , Ng. Brajachand Singh

Abstract:

Background:
HIV-TB co-infection continues to be a dual threat worldwide.

Aim and objectives:
To assess impact of ATT/ART treatment on CD4+T lymphocytes counts among HIV-TB co-infected patients

Material and methods:
A prospective longitudinal study on a total of 104 HIV and TB co-infected patients was conducted from November, 2017 to October, 2019, in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Hospital, Imphal, Manipur, India . Diagnosis of HIV was made as per NACO guidelines, Govt. of India using rapid kit tests. Diagnosis of TB was made as per RNTCP guidelines, Govt. of India. Estimation of CD4 cell counts was carried out by FACS count machine. Follow up of cases done after six months of initiation of ATT/ART.

Result:
In this study, there were 104 co-infected patients of HIV–TB. Out of the 104 subjects, 67 (64.4%) were from the rural area and the remaining 37(35.6%) were from urban area. There were more of males (72, 69.2%) than the females (32, 30.8%). The maximum number of co-infected patients were 46(42.2%) in the age group of 35-45 years. There were 58 cases of pulmonary TB, 41 cases of extra pulmonary TB and 5 cases of both. All the patients were on ATT and ART therapy. The 2nd CD4 count (283.59 cells /mm3) estimations after six months of therapy increases as compared to that of 1st CD4 counts (181.90 cells /mm3) and found statistically significant (p value = 0.0001).

Conclusion:
In the present study, there was improvement in CD4 counts after the co-infected patients were put on six months ATT/ART therapy. However regular monitoring of CD4 counts is highly required to avoid from other opportunistic infections.

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Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. In India, TB is the number one killer of adults among all infectious diseases. Tuberculosis is the most common opportunistic infection amongst HIV infected individuals. It is a major cause of mortality among patients with HIV and poses a risk throughout the course of HIV disease, even after successful initiation of antiretroviral (ART) therapy. In India, 55-60% of AIDS cases reported had TB and TB is one of the leading causes of death in people living with HIV/AIDS (PLHA)[1]
According to the World Health Organization (WHO), around one third of the 36.9 million people living with HIV&AIDS worldwide are co-infected with TB.Among all TB cases, 8.6% were people living with HIV (PLHIV)[2].

The risk of developing tuberculosis (TB) is estimated to be between 16-27 times greater in people living with HIV than among those without HIV infection[2]. In a HIV and TB co-infected person, the immune response to TB bacilli increases HIV replication. As a result of the increase in

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number of viruses in the body, there is rapid progression of HIV infection. The viral load can increase by 6-7 folds[1]. As a result, there is rapid decline in CD4 count and patient starts developing symptoms of various opportunistic infections (OIs). Thus the health of the patient who has dual infection deteriorates much more rapidly than with a single infection.

HIV infection increases the risk of TB and TB accelerates the course of HIV disease with increased viral load, a fall in CD4 counts which may lead to increase in mortality [3,4]. HIV infected individuals with lower CD4+T cell counts are more susceptible to tuberculosis than those individuals with higher counts. A decrease in CD4 count leads to various opportunistic infections which may also in turn lead to mortality and morbidity.

Since Manipur, which lies in the north eastern corner of India, neighboring with Myanmar is highly endemic to HIV, such co-infection of HIV and TB is common. Hence the present study was undertaken with the aim to assess impact of six months ATT/ART treatment on CD4+T lymphocytes counts among HIV/TB co-infected patients to improve the quality of life of people living with HIV/AIDS (PLHA). This is the first original study report from Manipur, India.

Materials and methods

A prospective longitudinal study was conducted in the Department of Microbiology, Regional Institute of Medical Sciences (RIMS), Hospital, Imphal, Manipur, India over a period of two years (November, 2017 to October,2019). The study population consist of104ART naive Human Immunodeficiency Virus (HIV) and tuberculosis (TB) co-infected individuals/patients who are eligible for antiretroviral therapy (ART) and anti-tubercular therapy (ATT)

Inclusion criteria:

ART naïve, HIV/TB co-infected individuals above the age of 18 years who are eligible for ART and ATT.

Exclusion criteria:

Persons already on ART, children under 18 years of age and patients not willing to participate in the study.

The study was approved by the Institutional Research Ethics board of Regional Institute of Medical Sciences (RIMS), a tertiary Hospital, Imphal, Manipur, India. Demographic and clinical details regarding the HIV status, type of TB, CD4 counts and any other associated OIS were collected.

The different specimens were collected from different sources like ICTC (Integrated Counseling and Testing Centre), Department of Microbiology RIMS; FACS (Fluorescence activated cell sorter) Count centre. Department of Microbiology RIMS; Department of Respiratory Medicine, RIMS; Designated microscopy centre, RNTCP, RIMS; ARTCentre, RIMS; Dept. of Medicine, RIMS; Patients referred to RIMS by different NGOs and community health care centers working with HIV/AIDS and TB.

The following investigations were conducted for diagnosis:

i) Diagnosis of HIV infection

Pre-test and post test Counseling were made as per NACO guidelines, Govt. of India and written informed consent was taken. Diagnosis of HIV positivity was made by using rapid kit tests like COMB AIDS, TRISPOT, TRILINE as per NACO guidelines [5], Govt. of India.

ii) Diagnosis of Tuberculosis.

The diagnosis of pulmonary tuberculosis was made by sputum microscopic examination by Ziehl-Neelsen staining technique, X-ray chest and sputum culture whenever necessary. The diagnosis of extra pulmonary tuberculosis (EPTB) was based on feature suggestive of TB with supportive evidence like pleural/asciticfluid examination, lymph node biopsy and radiologic findings as per RNTCP guidelines [6].

iii) Estimation of CD4+ T lymphocytes

Estimation of CD4+ T lymphocyteswas carried out by FACS Count Machine[7] (Becton Dickinson Biosciences, USA).

Follow up:

Estimation of CD4+T lymphocyte count was conducted every 6 months as per NACO guidelines[7,8] during the study period. Also regular ART and ATT were ensured.

Statistical analysis:

Data entry and analysis were performed using SPSS version-16 software. Different characteristics of study participants were described using percentage, range and mean as appropriate. Significance was tested by using Pearson's Chi-square test, t-test and Spearman's correlation coefficient test. P-value <0.05 was considered as significant.

Results

A total of 104 clinically and laboratory confirmed cases of HIV-TB co-infected patients participated in this study. The socio-demographic

profiles of the subjects were studied. The district wise distribution of the one hundred and four subjects with their percentage is shown in table 1.Out of the 104 subjects, there were 67(64.4%) rural and 37(35.6%) urban distribution of HIV-TB co-infected subjects. There were 32 (30.8%) females and 72 (69.2%) males in the study group. The maximum number of co-infected patients were 46(44.2%) in the age range of 35-44 years and the least 1(0.96%)each in the age categories of 55-64years and 75-84 years as shown in fig.1. There were 58 cases of pulmonary TB(PTB), 41 cases of extra pulmonary TB (EPTB) and 5 cases of both PTB with EPTB as shown in table2. Among the 41 cases of EPTB, lymph node TB consist of 31 and 5 each casesof abdominal and bone TB .

The change of the CD4 levels at the base line (1st CD4) and that at six months (2nd CD4) of the respective treatment (ART or ART with ATT) were observed. Outcome of the subjects after regular treatment during the study period, 15(14.4%) subjects expired and 3 (2.9%) defaulted. All the rest 86 (82.7%) survived as shown table 2. The 15 expired subjects had low CD4 count (<100/cu.mm.) with a minimum of 18/cu.mm.Regarding the ATT type of distribution, 68 subjects(65.4%) were on DOTS and the remaining 36(34.6%) were on non DOTS (table 2).

The sex wise mean CD4 counts at the initiation of ART therapy (1st CD4) in females and males were 225.71cells /$mm^3$and 162.43 71cells /mm^3 respectively. The mean CD4 counts after 6 month of therapy (2nd CD4) in females and males were 336.09cells/$mm^3$and 255.03 cells /mm^3 respectively as shown in table 3 and fig.2. The standard deviation of the 1st CD4 and 2nd CD4 sex wise category were also calculated. The significance of the sex wise difference was statistically found to be significant since the p value is less than 0.05. The 1st and 2nd CD4 count estimations after six months of ART therapy shows an increased in the 2ndCD4 counts (283.59cells /mm3) as compared to that of 1st CD4 counts (181.90cells /mm^3) as shown in table3, fig2and found significant (p value = 0.0001). The significant improvements in the CD4 counts were also seen in the age categories of 25-34 year, 35-44years and 45 -54years as the p value were less than 0.05(p=0.003,p=0.002and p=0.001 respectively).

Table 1.District wise distribution of the subjects

Sl .no	Districts	Subjects	Percentage
1	Bishnupur	12	11.5
2	Churachandpur	1	1.0
3	Chandel	6	5.8
4	Imphal East	11	10.6
5	Imphal West	35	33.7
6	Jiribam	1	1.0
7	Senapati	18	17.3
8	Tamenglong	1	1.0
9	Thoubal	9	8.7
10	Ukhrul	10	9.6
	Total	104	100.0

Table2.Type of TB, subjects on DOTS, Non DOTS and outcome of the subjects after regular treatment during the study.

Sl no.	Parameter	Number of subjects during the study with %
1	Pulmonary TB (PTB)	58 (55.8)
2	Extra pulmonary TB (EPTB)	41 (39.4)
3	PTB+EPTB	5 (4.8)
	Total	104 (100)
4	Subjects under DOTS	68 (65.4)
5	Subjects under Non DOTS	36 (34.6)
	Total	104 (100)
6	Default	3 (2.9)
7	Expired	15 (14.4)
8	Surviving	86 (82.7)
	Total	104 (100)

**DOTS: Directly observed treatment short course*

Table3.Comparison of improvement of 1st CD4 and 2nd CD4 after 6months of ART/ATT in respect of sex wise distribution and their total mean value

Sex		1st CD4 (cells /mm^3)	2nd CD4 (cells /mm^3)
Female (n=50)	Mean	225.71	336.09
	Std. dev.	174.32	180.98
Male (n=54)	Mean	162.43	255.03
	Std. dev.	134.60	177.40
Total	Mean	181.90	283.59
	Std. dev.	149.99	181.84

Discussion

Tuberculosis, caused by Mycobacteriu-mtuberculosis, remains an important public health challenge and has been worsened by the HIV epidemic, resulting in an increased of morbidity and mortality worldwide.

The primary impact of HIV on TB is that the risk of developing TB becomes higher in patients with HIV. An HIV infected person, newly infected with TB has 10-30 times higher chances of developing the disease than among patients infected with TB only. Amongst PHLA, 3-10% of persons develop TB per year [1]. Further more, HIV-infected persons with TB suffer much higher mortality than non-HIV infected persons. Even if TB is successfully treated, long term post-TB mortality among PLHA is extremely high.

Fig.1. Age wise distribution of HIV –TB co-infection

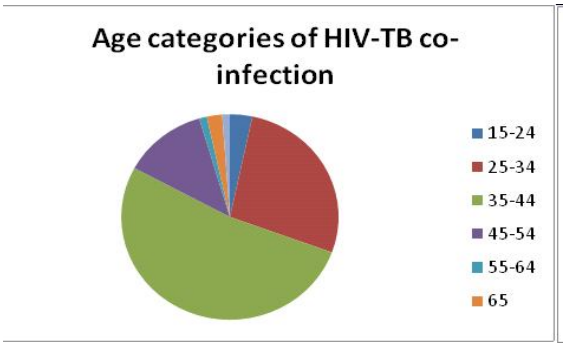
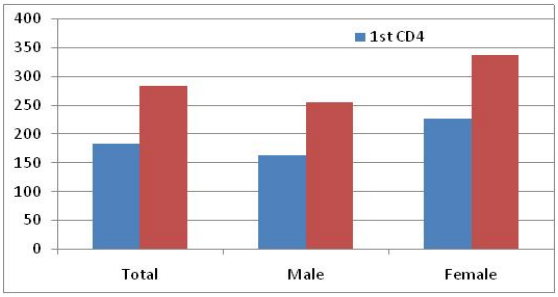


Fig.2. Means of 1st CD4 and 2nd CD4 in respect of the total and sex wise.



In a TB-HIV co-infected person, the immune response to TB bacilli increases HIV replication. As a result of the increase in number of viruses in the body, there is rapid progression of HIV infection. The viral load can increase by 6-7 folds[1]. As a result, there is a rapid decline in CD4 count and patient starts developing symptoms of various opportunistic infections. Thus the health of the patient who has dual infection deteriorate more rapidly than with a single infection.

The mechanism involved in the progression and the impact of HIV-TB co-infection on host's immune system has not been understood com¬pletely. The pathophysiological processes of TB involve both innate and cell mediated immunity. The innate immune responses involve the activity of macrophages and dendritic cells (DC) whereas the cell mediated immunity involves the predominance of T lymphocytes. The prime targets for tuberculosis are macrophages present in the alveoli[9]. HIV infection causes a rapid decline of immune responses resulting in multiplication of the mycobacterium within the granuloma, an organized structure comprising epitheloid macrophages surrounded by a rim of lymphocytes, to restrain tubercle bacilli, leading to their eventual multiplication and dissemination, thereby culminating in severe pathology. The killing of CD4+ T cells by HIV within granuloma is associated with primary tuberculosis or reactivation of tuberculosis [10,11]. It is emphasized that there will be an increased replication of HIV at the sites of mycobacterium infection by multiplying within the activated CD4 + T cells and the macrophages accumulating at the site of granuloma. The death of CD4 + T cells within the granuloma also causes the reactivation of the infection. Thus HIV infected individuals with lower CD4 counts are more suscep¬tible for attaining TB rather than individual with higher CD4 counts[12]. In vitro studies conducted by various workers demonstrate that the pro-inflammatory cytokines such as TNF α , IL 1 β, and IL 6 produced by the phagocytosis of mycobacterium aid in the multiplication of HIV- 1. Thus HIV-TB co-infection is associated with reduced survivability of macrophages and elevated pro-inflammatory cytokines creates a symbiotic environment for the coexistence of both the pathogens [9,12]. Pathak et al[13] reportedthat the co-infection of HIV-1/TB synergistically decreased the viability of macrophages and increased levels of pro - inflammatory cytokines and this seemed to be specific to M. tuberculosis rather than other species of mycobacteria.

In the present study, the majority of the study subjects were more concentrated in the Imphal west district of Manipur, India, which is the most developed and thickly populated area of the state. Out of the 104 subjects, 67 (64.4 %) were from rural areas and 37 (35.6%) from urban area. Hence most of the study subjects were from rural area. A similar finding was also reported in a study in southern India by Kamath et al.[14]. The majority of the subjects were in the age group of 25-44 years.This is in consistent with the findings conducted by Kamath et al[14] and Mundhra et al [15]. There were more males with 72 (69.2%) than females having 32 (30.8%) in the sex distribution which is also in consistent with the findings of Kamath et al [14] and Mundhra et al [15]. This male predominance may be due to their migration for employments within and outside the state, thereby exposing to high risk behavior and presence of other OIs at the time of diagnosis. There were 15 (14.4%) mortalities during the study. Out of which fourteen were males and one was female. Of the fifteen mortalities, two male subjects were suffering from type 2 diabetes mellitus. The importance of diabetes mellitus as co-morbidity of TB in addition to HIV was studied by Gupta et al[16]. The three male subjects defaulted (2.9%) were from rural areas. The probable reason may be due to long distances to be travelled and centralization of the health care facilities.

There were more 58 PTB(55.8%) than 41 EPTB

(39.4%) and there were only 5 subjects of both PTB&EPTB(4.8%) in the present study. There were 68 subjects on DOTS (65.4%) and 36 on non DOTS (34.6%).

At initial presentation (1st CD4), the mean CD4 count was 181.90 cells /mm^3, following six months of therapy (2nd CD4) which increased to 283.59cells /mm^3. The results showimprovement of the CD4 levels. It was found to be statistically significant since p value was <0.05 (0.000 1). A similar finding wasalso reported by Kavya et al [17].

Conclusions

HIV-TB co-infection continues to be a major dual public health challenge worldwide. In our present study, we found that there was significant recovery of CD4 cell counts among HIV –TB co-infected patients after six month of initiation of ATT/ART. A decrease in CD4 count leads to various opportunistic infections which in turn lead to morbidity and mortality. Improvement in the CD4 count will protect against many other opportunistic infections thereby reducing morbidity and mortality which may increase the life span of patients qualitatively and quantitatively. Hence, regular CD4count monitoring is highly essential for patients before initiation of ATT/ART which can reduces the complications associated with the dual infection of HIV and TB.

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Conflicts of interest

There are no conflicts of interest.

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