Original article

COMPARISON OF CD4 AND CD8 COUNTS IN HIV NEGATIVE PULMONARY TUBERCULOSIS PATIENTS WITH NORMAL HEALTHY CONTROLS IN AND AROUND PRAYAGRAJ

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ABSTRACT

Anti-tuberculosis immunity involves a cellular immune response for their control. A critical marker of immunologic integrity is the CD4 and CD8 cell counts. Tuberculosis may be a cause of non-HIV associated CD4 and CD8-T cell lymphopenia. This study compares mean CD4, CD8 cell count and CD4:CD8 Ratio in pulmonary tuberculosis patients never had treatment for tuberculosis, pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month and normal healthy controls. A case control study done in Prayagraj from October 2019 to October 2020 includes HIV negative, sputum positive pulmonary tuberculosis patients never had treatment for tuberculosis(n=25), pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month(n=24), and normal healthy controls(n=36). We collected details including age, sex, symptoms of pulmonary tuberculosis, anti-tuberculosis treatment and investigated for HIV testing by ELISA, Sputum for AFB, Sputum for CBNAAT, CD4 and CD8 cell count determined by flow cytometrically.

The mean CD4 and CD8 cell count was significantly lower in HIV negative pulmonary tuberculosis patients never had treatment for tuberculosis than in normal healthy controls (p value<0.001) and CD4:CD8 ratio also lower (p value=0.013). The mean CD4 and CD8 cell count higher in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month than in pulmonary tuberculosis patients never had treatment for tuberculosis (p value<0.001) and CD4:CD8 ratio also higher (p value=0.013). CD4 and CD8 lymphopenia is an acceptable phenomenon in HIV negative pulmonary tuberculosis patients and such lymphopenia improves with anti-tuberculosis drug regimens as per protocol. This study highlights the importance of CD4, CD8 cellular immune response conducted by T- lymphocytes in outcome of pulmonary tuberculosis.

Key Words: Pulmonary tuberculosis, CD4 count, ELISA, CBNAAT

INTRODUCTION

Tuberculosis has been a major cause of suffering and deaths since ancient times. The history of tuberculosis is old as the mankind.[1] It is one of the top 10 causes of death and the leading cause from a single infectious agent (Mycobacterium tuberculosis), ranking above HIV/AIDS. The disease can affect anyone, anywhere, but most people who develop tuberculosis (about 90%) are adults, the male: female ratio is 2:1. Almost 90 % of cases each year are in 30 high tuberculosis burden countries.[2] An estimated 10.0 million (range 9.0-11.1 million) people fell ill with tuberculosis in 2018,

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a number that has been relatively stable in recent years.[2] In 2018, India was able to achieve a total notification of 21.5 lakh tuberculosis cases of which 25% was from the private sector.[3] Uttar Pradesh, with 17% of population of the country, is the largest contributor to the tuberculosis cases in with 20% of the total notifications, accounting to about 4.2 lakh cases (187 cases per lakh population).[3]

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (MTB). Rising trend in HIV infection in some countries together with the emergence of multi-drug resistant (MDR) strains of tuberculosis pose an additional threat.[4]

Anti-tuberculosis immunity involves innate as well as adaptive immunity at various levels following *Mycobacterium tuberculosis* infection. Anti-tuberculosis immunity involves a cellular immune response for their control. A critical marker of immunologic integrity is the CD4 and CD8 cell counts. CD4+T helper lymphocytes play a central role in regulation of immune response .[5] They have capacity to help B cells for generating antibodies, to recruit and activate macrophages, to recruit neutrophils, eosinophils and basophils to sites of infection and inflammation.[6] Patients with tuberculosis manifest significant immunologic abnormalities, including anergy and failure of T lymphocytes to proliferate and produce INF-γ in response to mycobacterial antigens.[7]

A critical marker of immunologic integrity is the CD4 cell counts. It has been shown that CD4+ T-lymphocytes are most important in protective response against mycobacterium tuberculosis. In murine studies, T-cell deficiency was associated with increased susceptibility to disease.[8-10] CD8+ T-lymphocytes are also important for effective T-cell immune response against *Mycobacterium tuberculosis* infection, forming a cuff at the periphery of epithelioid cell granulomas.[11] They are capable of secreting cytokines such as INF-γ and IL-4 and thus may play a role in regulating the balance of Th-1 and Th-2 cells in the lungs of patients with pulmonary tuberculosis.[8]

The present study was carried out in department of pulmonary medicine Swaroop Rani Nehru Hospital Prayagraj to look at the occurrence of CD4 and CD8 cell lymphopenia in HIV negative pulmonary tuberculosis patients and compared to normal healthy controls.

METHODOLOGY

The present study was conducted in Department of Pulmonary Medicine Swaroop Rani Nehru Hospital Prayagraj Uttar Pradesh India from October 2019 to October 2020. It was a case control study. Institutional ethics committee MLN Medical College Prayagraj grant the permission prior to the start of the study, meeting held on 05-10-2019.

The following groups of subjects were included in the study after a written informed consent.

Case-Previous ATT-: A total of 25 patients with negative serology for HIV and sputum positive pulmonary tuberculosis who never had treatment for tuberculosis were registered in our study.

Case-Previous ATT+: A total of 24 patients with negative serology for HIV and sputum positive pulmonary tuberculosis had received anti-tuberculosis treatment for more than one month.

Control: A total of 36 normal healthy controls of ethnically matched age, sex who had never been treated for any form of tuberculosis and negative serology for HIV.

All subjects were enrolled in this study as per inclusion and exclusion criteria and detailed history was recorded and routine investigations were done.

All subjects were investigated for following parameters:

1. HIV testing ELISA
2. Sputum smear for AFB
3. Sputum for CBNAAT
4. CD4 cell count by flow cytometer
5. CD8 cell count by flow cytometer

And ratio of CD4 and CD8 also estimated and recorded accordingly.

Inclusion Criteria:

- Subject with negative serology for HIV.
- Pulmonary tuberculosis case confirmed by sputum smear microscopy positive for Acid Fast Bacillus (AFB) or pulmonary tuberculosis case confirmed by sputum for CBNAAT (Cartridge Based Nucleic Acid Amplification test).
- Apparently healthy control with sputum for AFB

negative.

- Age Greater than 18 year.
- Patient / guardian giving informed consent.

Exclusion Criteria:

Patients having any of the following conditions were excluded from the study:

- Consent not given by patient / guardian.
- Seropositive for HIV.
- Chronic illness like diabetes, chronic liver disease, chronic kidney disease or any other comorbid condition.
- History of alcohol intake.
- History of smoking, tobacco or other addiction.
- Patients on steroid or cytotoxic drugs.
- Concurrent use of immunosuppressant.
- Other immunocompromised patients.
- Age less than 18 years.

HIV testing by ELISA:

2-3 ml blood collected aseptically in a clean sterile tube for HIV testing after counselling and obtaining due informed consent. In MLN Medical College the collected sample was tested for HIV antibody (HIV1/HIV2) using rapid diagnostic kit following the NACO, India guidelines.

Sputum smear for Acid Fast Bacillus (AFB):

Two sputum samples were collected in sterile leak proof, disposable, appropriately labeled containers without any fixative as per RNTCP guidelines. In MLN Medical College the collected specimens were subjected for demonstration for Acid Fast Bacilli (AFB) using fluorescent microscopy employing Auramine O stain and grading was done accordingly. The sputum smear microscopy has a sensitivity of 64% and specificity of 98%.

Sputum for CBNAAT:

In MLN Medical College sputum for CBNAAT is a new diagnostic test cartridge based nucleic acid amplification test was done. CBNAAT was rapid, fully automated based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimen and also detects rifampicin resistance. This diagnostic test was designed to purify, concentrate, amplify and identify targeted rpoB nucleic acid sequences and delivered the result in about 2 hours.

Measurement of CD4 and CD8 T-cell count determination by flow cytometer:

CD4 and CD8 level were measured by taking blood sample in EDTA vacutainers and processed on a flow cytometer using true count tubes with beads and tri test (CD3, CD4, CD8 Cocktail) antibody following a lyse-no-wash protocol. CD4 cell count of 381-1170 cells/µl, CD8 cell count of 108-845 cells/µl, and CD4: CD8 Ratio of 0.55 to 3.03 were considered to be normal range as per manufacturer's instructions.

Analysis of Data:

Data were coded and recorded in Microsoft excel spreadsheet program. SPSS v23 (IBM Corp.) was used for data analysis. Descriptive statistics were elaborated in the form of means/standard deviations and medians/ IQRs for continuous variables and frequencies and percentages for categorical variables. Data were presented in a graphical manner wherever appropriate for data visualization using histograms/box and whisker plots/column charts for continuous data and bar charts/pie charts for categorical data. Group comparisons for continuously distributed data were made using independent sample 't' test when comparing two groups. If data were found to be non-normally distributed, appropriate non-parametric test in the form of Wilcoxon test were used. Chi-squared test was used for group comparisons for categorical data. In case the expected frequency in the contingency tables was found to be <5 or >25% of the cells, Fisher's Exact test was used instead. Linear correlation between two continuous variables was explored using Pearson's correlation (if the data were normally distributed) and Spearman's correlation (for non-normally distributed data). Statistical significance was kept at $p<0.05$.

RESULTS

The mean age of Case Previous ATT- group was 37.48 (±14.54) years and mean age of Case Previous ATT+ group was 29.08 (±12.18) years and mean age of Control group was 32.86 (±9.73) years.

18 males (72%) and 7 females (28%) in Case Previous ATT- group, 15 males (62.5%) and 9 females (37.5%) in Case Previous ATT+ group, 23 males (63.9%) and 13 females (36.1%) in Control

Table 1: Comparison of the 3 Subgroups of the Variable Subgroup in Terms of CD4 Count (n = 85)					
CD4 Count (cells/µl)	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	χ^2	p value
Mean (SD)	375.20 (±115.84)	567.71 (±191.63)	748.89 (±176.61)	43.148	<0.001
Median (IQR)	380 (304-437)	538.5 (434.75-612.25)	767.5 (646.5-873)		
Range	80 – 595	298 – 983	364 - 1200		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.003	
Case-Previous ATT- - Control				<0.001	
Case-Previous ATT+ - Control				0.009	

group.

Case- Previous ATT-: Never had treatment for tuberculosis.

Case- Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD4 Count was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD4 Count in the Case-Previous ATT- group was lower than the mean (SD) of CD4 Count in the Case-Previous ATT+ group. The mean (SD) of CD4 Count in the Case-Previous ATT+ group was lower than the mean (SD) of CD4 Count in the Control group. The median (IQR) of CD4 Count in the Case-Previous ATT- group was lower than the median (IQR) of CD4 Count in the Case-Previous ATT+ group. The median (IQR) of CD4 Count in the Case-Previous ATT+ group was lower than the median (IQR) of CD4 Count in the Control group. The ranged CD4 Count in the Case-Previous ATT- group was lower than the ranged CD4 Count in the Case-Previous ATT+ group. The ranged CD4 Count in the Case-Previous ATT+ group was lower than the ranged CD4 Count in the Control group.

There was a significant difference between the 3 groups in terms of CD4 Count (χ^2 = 43.148, p = <0.001), with the median CD4 Count being highest in the Control group. **Case-Previous ATT-: Never had treatment for tuberculosis.**

Case-Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD8 Count was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD8 Count in the Case-Previous ATT- group was lower than the mean (SD) of CD8 Count in the Case-Previous ATT+ group. The mean (SD) of CD8 Count in the Case-Previous ATT+ group was lower than the mean (SD) of CD8 Count in the Control group. The median (IQR) of

Table 2: Comparison of the 3 Subgroups of the Variable Subgroup in Terms of CD8 Count (n = 85)					
CD8 Count (cells/µl)	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	χ^2	p value
Mean (SD)	337.96 (±119.61)	407.08 (±217.53)	555.22 (±157.57)	26.003	<0.001
Median (IQR)	320 (279-392)	335.5 (267.25-469.5)	558.5 (426.75-630.75)		
Range	141 - 637	176 - 1170	209 - 891		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.661	
Case-Previous ATT- - Control				<0.001	
Case-Previous ATT+ - Control				<0.001	

Table 3: Comparison of the 3 Subgroups of the Variable Subgroup in Terms of CD4:CD8 Ratio (n = 85)					
CD4:CD8 Ratio	Subgroup			Kruskal Wallis Test	
	Case-Previous ATT-	Case-Previous ATT+	Control	$\chi 2$	p value
Mean (SD)	1.17 (±0.39)	1.62 (±0.68)	1.43 (±0.52)	8.756	0.013
Median (IQR)	1.09 (0.94-1.46)	1.71 (1.09-1.99)	1.24 (1.2-1.54)		
Range	0.57 - 2.06	0.61 - 3.26	0.98 - 3.37		
Pairwise Comparison of Subcategories of Subgroup				Adjusted P Value	
Case-Previous ATT- - Case-Previous ATT+				0.011	
Case-Previous ATT- - Control				0.111	
Case-Previous ATT+ - Control				0.617	

CD8 Count in the Case-Previous ATT- group was lower than the median (IQR) of CD8 Count in the Case-Previous ATT+ group. The median (IQR) of CD8 Count in the Case-Previous ATT+ group was lower than the median (IQR) of CD8 Count in the Control group. The ranged CD8 Count in the Case-Previous ATT- group was lower than the ranged CD8 Count in the Case-Previous ATT+ group. The ranged CD8 Count in the Case-Previous ATT+ group was nearly same the ranged CD8 Count in the Control group.

There was a significant difference between the 3 groups in terms of CD8 Count ($\chi 2 = 26.003$, $p = <0.001$), with the median CD8 Count being highest in the Control group.

Case-PreviousATT-: Never had treatment for tuberculosis.

Case-Previous ATT+: Received anti-tuberculosis treatment for more than one month.

Control: Normal healthy controls.

The variable CD4:CD8 Ratio was not normally distributed in the 3 subgroups of the variable Subgroup. Thus, non-parametric test (Kruskal Wallis Test) was used to make group comparisons.

The mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The mean (SD) of CD4:CD8 Ratio in the Control group was lower than the mean (SD) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT+ group. The median (IQR) of CD4:CD8 Ratio in the Control group was lower than the median (IQR) of CD4:CD8 Ratio in the Case-Previous ATT+ group.

The ranged CD4:CD8 Ratio in the Case-Previous ATT- group was lower than the ranged CD4:CD8 Ratio in the Case-Previous ATT+ group. The ranged CD4:CD8 Ratio in the Case-Previous ATT+ group was lower than the ranged CD4:CD8 Ratio in the Control group.

There was a significant difference between the 3 groups in terms of CD4:CD8 Ratio ($\chi 2 = 8.756$, $p = 0.013$), with the median CD4:CD8 Ratio being highest in the Case-Previous ATT+ group.

DISCUSSION

The present study was conducted in Department of Pulmonary Medicine, Swaroop Rani Nehru Hospital Prayagraj from October 2019 to October 2020. A total of 85 candidates were selected for the study who fulfilled the inclusion and exclusion criteria and gave consent for the study. Among 85 candidates, 49 were HIV negative pulmonary tuberculosis patients (sputum smear microscopy positive for AFB or sputum for CBNAAT) and 36 were normal healthy controls. Of these 49 HIV negative pulmonary tuberculosis patients, 25 pulmonary tuberculosis patients never had treatment for tuberculosis and 24 pulmonary tuberculosis patients had received anti-tuberculosis treatment for more than one month. There were no significant difference between mean age (p value=0.637) and sex distribution (p value=0.74) in HIV negative pulmonary tuberculosis patients and in normal healthy controls. The CD4 cell count and CD8 cell count of the respective sample were recorded in all the candidates. The ratio of CD4 and CD8 also estimated and recorded accordingly.

Comparison of the Variable subgroups in terms of CD4 count (n=85)

In our study we found CD4 cell count was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Uppal**

et al [12], **Sabhapandit et al** [13] and **Siraj et al** [14] also found CD4 cell count was lower in patients of pulmonary tuberculosis compared to healthy controls. In our study we also found that mean CD4 cell count was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month.

From our study and previous studies this can be predicted that pulmonary tuberculosis is responsible for CD4-T Cell lymphopenia.

Comparison of the Variable subgroups in terms of CD8 count (n=85)

In our study we found CD8 cell count was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Sabhapandit et al** [13] and **Siraj et al** [14] also found CD8 cell count was lower in patients of pulmonary tuberculosis compared to healthy controls. In our study we also found that mean CD8 cell count was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month.

From our study and previous studies this can be predicted that pulmonary tuberculosis is responsible for CD8-T Cell lymphopenia.

Comparison of the Variable subgroups in terms of CD4: CD8 Ratio (n=85)

In our study we found CD4:CD8 Ratio was lower in HIV negative pulmonary tuberculosis patients than in normal healthy controls. Similarly, **Uppal et al** [12] and **Siraj et al** [14] also found CD4:CD8 Ratio was lower in patients of pulmonary tuberculosis compared to healthy controls. This finding is contrary to that observed by **Sabhapandit et al** [13] In our study we also found that mean CD4:CD8 Ratio was lower in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month. From our study and previous studies this can be predicted that pulmonary tuberculosis may be responsible for change in CD4:CD8 Ratio.

CONCLUSION

In conclusion from our study found significantly lower mean CD4 and CD8 cell count and CD4:CD8 ratio among HIV negative pulmonary tuberculosis patients compared with normal healthy controls. We also found lower mean CD4 and CD8 cell count and CD4:CD8 ratio in pulmonary tuberculosis patients who never had treatment for tuberculosis than in pulmonary tuberculosis patients who have received anti-tuberculosis treatment for more than one month. According to our study results patients of pulmonary tuberculosis had lower number of both CD4 and CD8 cell count than normal healthy controls which can be a sign of suppressed cellular immunity in pulmonary tuberculosis patients. This study highlights the importance of cellular immunity, conducted by T-lymphocytes in outcome of pulmonary tuberculosis.

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CONFLICT OF INTEREST

None

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