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Medicine

Comparison of CD4+ T lymphocyte counts in HIV negative patients of pulmonary tuberculosis and tuberculous meningitis

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ABSTRACT

Background

The co-relation between HIV and CD4+ T lymphocyte counts has been extensively studied over the past 3 decades. The significance of CD4+ lymphopenia in other diseases has been studied less exhaustively. We have evaluated the CD4+ counts and nutritional status in patients of pulmonary and extra pulmonary tuberculosis, in the absence of HIV infection.

Methods

Patients diagnosed with pulmonary and extra pulmonary TB (tuberculous meningitis) were recruited from the in-patient wards over a 1 year period at a tertiary centre in Dehradun. PTB was diagnosed on the basis of sputum smear positivity for AFB, and TBM was diagnosed on clinical grounds, and/ or CSF examination and response to ATT. After excluding HIV infection in all patients, CD4+ counts were measured using flow cytometry. The counts and the nutritional status of the 2 groups of patients were then compared using chi square analysis with the help of SPSS 23.0.

Results

In total, 82 patients were recruited, 50 patients suffering from PTB and 32 suffering from TBM. There was a male dominance with majority patients below the age of 30 years in both groups of patients. The mean CD4+ counts in the PTB group were 541.54 cells/mm^3 with 34% of the patients having subnormal counts. The mean CD4+ counts in the TBM group were 481.75 cells/ mm^3 with 53.12% of the patients having subnormal counts. The mean BMI in the 2 groups was 22.95kg/m^2 and 21.85 kg/ $m^{2.}$., with 12% patients underweight in the PTB group and 25% underweight in the TBM group. Increase in BMI was associated with higher CD4+ counts in both sets of patients.

Conclusions

A high proportion of tuberculosis patients had subnormal counts despite the absence of HIV infection. The mean CD4+ counts were significantly lower in the TBM group as compared to the PTB group, though both groups had counts significantly lower than the normal population. Lower BMI was associated with lower CD4+ counts, which progressively increased with increase in BMI. The improvement in CD4+ count, if any, to ATT could however not be assessed by this study.

KEYWORDS :

Introduction

Tuberculosis, despite being one of the oldest known diseases to mankind, remains one of the leading cause of morbidity and mortality worldwide, especially in developing nations (1). Approximately 1.8 billion people in the world are infected with tubercular bacilli. The WHO estimated 9.5 million new cases of TB (all forms, pulmonary and extra-pulmonary) occur every year. Another 2 million deaths from TB are estimated every year. 95% of these cases and deaths are reported from developing countries. Southern parts of the African continent, along with the Indian sub-continent have the highest prevalence and incidence of tuberculosis(2). The 6 countries that stand out as having the largest number of incident cases in 2014 were India, Indonesia, Nigeria, Pakistan, People's Republic of China and South Africa(3). Though India is the second-most populous country in the world, one fourth of the global incident TB cases occur in India annually. As per WHO Global TB Report, 2015, out of the estimated global annual incidence of 9.6 million TB cases, 2.2 million were estimated to have occurred in India. The prevalence of HIV – TB co-infection, and MDR TB has started to increase on an alarming basis.

The correlation between HIV and CD4+ counts has extensively been studied over the past 3 decades. The counts play an important role in diagnosis, prognosis, and studying progression and deciding ART in patients of HIV[(4)]. The normal adult CD4 count for most laboratories falls in a range of 800 to 1050 cells/mm^3; furthermore, when considering laboratory variations of two standard deviations, the normal CD^4 cell count range falls within 500 to 1400 cells/mm3 [(5)]. A CD4 count of <200 cells/mm^3 indicates the clinical stage of AIDS, which implies a high susceptibility to opportunistic infections In contrast, these counts have been less extensively studied in other diseases including TB. Recent studies over the past decade or so have shown decreased CD4+ counts in patients of TB, despite the absence of HIV [(6)]. The relevance of this phenomenon to the pathogenesis of tuberculosis is unknown, although it appears to be a secondary effect, rather than a pre-disposing cause, but it could in part explain as to why tuberculosis acts in synergy with HIV to reduce the host's immune competence (7) An increase in these counts in response to successful ATT has also been sporadically observed (8). The improvement of nutritional status has also been shown to improve the CD4+ counts in patients of tuberculosis (9). It is currently unknown whether the CD+ lymphopenia is a consequence of the MTB infection in patients of TB, or if pre-existing CD4+ lymphopenia in these patients predisposes to MTB infection, even in the absence of HIV.

In this study we investigated CD4 counts in HIV negative patients with pulmonary and extra pulmonary tuberculosis. The hypothesis of this study was that CD4+ are lower in patients of Tuberculosis even in the absence of HIV infection, and that lower CD4+ T lymphocytes counts pre-dispose to more severe forms of tuberculosis with an overall higher morbidity and mortality rate. We also evaluated and compared the CD4+ T lymphocyte counts and the nutrition status in patients of pulmonary tuberculosis, with those suffering from tubercular meningitis.

Materials and Methods

Ethics statement

All subjects were provided written detailed informed consent prior to inclusion into the study. In cases where the patients were unable to give informed consent on account of altered sensorium, the closest relatives were encouraged to give informed consent. No interventions interfering with standard care or diagnosis were done, with the exception of CD4+ counts. The study was approved by the ethics committee of the concerned institute.

Study design and setting

The study was conducted in the Department of Medicine, Himalayan

Institute of Medical Sciences, Swami Ram Nagar, Dehradun, over a period of 12 months. Subjects were recruited from the in patients from the department of medicine.

Type of study : Observational & analytical, cross sectional study

Sample size : 82 patients were recruited, which included 50 patients of pulmonary tuberculosis and 32 patients of tubercular meningitis.

Selection of subjects

Diagnosis of PTB was made on the basis of sputum smear positivity for AFB on the basis of RNTCP guidelines. Diagnosis of TBM was based on compatible clinical features and CSF picture, microbiological and/ or radiological features and a positive response to anti-TB therapy. Patients unwilling to give consent, having HIV infection, or those on immunosuppressive medications were excluded from the study.

Study protocol

Assessment of nutritional status was done by calculation of BMI from the weight and height of the patients. Baseline investigations included complete haemogram, liver and kidney function tests, random blood sugar and chest x ray. Sputum for AFB was done for all patients of PTB. HIV serology was done for all patients after taking informed voluntary consent. CSF and radiological examination of the head was done wherever indicated for the diagnosis of TBM.

CD4+ T lymphocyte count analysis by flow cytometry was done for all patients. The machine used was Navios – 8 colour flow cytometry manufactured by Beckman coulter. 10 microlitres of centrifuged samples of the patient were required by the above mentioned flow cytometry machine to estimate the counts. Counts below 500 cells/ mm3 were deemed sub-normal.

Data Management and Statistical Analysis

Data were analyzed using SPSS software version 23.0 Descriptive statistics involved the use of proportions, percentages and means as appropriate. Comparison of mean CD4 counts was done using Student's t –test. The associations between categorical variables was analyzed using the Chi-squared test. A 'p' value of less than 0.05 was considered statistically significant.

Results

Mean age of patients was 45.88 years for PTB and 42.43 years for TBM with an age range of 11 to 88 years (Table 1). Males slightly outnumbered females in both groups(Table 2).

Mean BMI was 22.95 kg/m^2 in PTB and 21.81 kg/m^2 in TBM group (Table 3). 25.00% patients of TBM were underweight as compared to 12.00% of the PTB patients.

The mean CD4+ count in the PTB group was 541.54cells/ mm^3 and in the TBM group was 481.75 cells/ mm^3 (Table 4). The counts were significantly lower in the extra pulmonary group (p= 0.031). In both sets of patients, the CD4+ counts were lower than the accepted lower limits of normal values despite the absence of HIV infection. The CD4+ counts progressively increased as the BMI increased in both sets of patients. In patients of PTB, an increase in BMI lead to a statistically significant increase in CD4+ counts (Table 5). Similar trends were observed in the TBM group (Table 6).

The mean CD4+ count of cases with 3+ sputum (412.5 cells/ mm^3) was significantly lower than 1+sputum (599.0 cells/ mm^3) positive (p=0.037) (Table 7). However, no relationship between grading of sputum positivity and BMI could be established.

Discussion

In this study, we attempted to find a co-relation between CD4+ counts and nutritional status of patients with types and severity of TB. We studied 82 patients of TB, 50 of which suffered from Pulmonary Tuberculosis (PTB), and 32 from Tubercular Meningitis (TBM). HIV was excluded in all patients to rule out the most apparent cause of CD4+ lymphopenia (if any). The nutritional status of these patients was assessed via Body Mass Index (BMI), and CD4+ counts were measured using flow cytometry.

The mean age of the 50 patients of PTB was 45.88 years with an age range of 11 to 88 years. The mean age of the 32 patients of TBM was 42.48 with an age range of 14 to 76 years. 34.38% of the patients were below the age of 30 years. The maximum incidence of patients below the age of 30 years in both sets of patients are in agreement with various studies which have showed that in the developing world, TB rates are highest among young adults, reflecting primary transmission in this age group (10). 54% of the patients in the PTB group and 59.38% in the TBM group were males, hence outnumbering females in both the groups. This is in agreement with the longstanding observation thought to reflect more frequent TB exposure in the community among men than women. The reason for these differences are thought to be social, rather than biological. Male dominance in most studies may be attributable to the fact that men are more mobile and hence have more exposure to the causative organism in the community.

The mean BMI in PTB group was 22.95±5.20 kg/m^2 and in TBM group was 21.81±4.97 kg/ m^2. In the PTB group, 12.00% of the patients were underweight (BMI less than 18.5kg/m^2) and 38.00% of the patients were overweight (BMI more than 23.5 kg/m^2). In the TBM group, 25.00% of the patients were underweight and 21.87% of the patients were overweight, these findings being consistent with other similar studies (11) . While the overall 'p' value of BMI between the two groups of patients was not significant, 25.00% of the TBM patients were underweight as compared to only 12.00% of the PTB patients. Similarly only 21.87% of the patients were overweight in the TBM group as compared to 38.00% in the PTB group.

Next, the CD4+ counts of the 2 groups of patients were measured and evaluated. It has been established that even in the absence of HIV infection, patients of TB have lower CD4+ counts than the general population (12). It has also been established, to a lesser extent, that the CD4+ counts in patients of TB improve marginally after effective ATT, but often do not reach the normal levels (9). In this study, the mean CD4+ count in 50 patients of PTB was 541.56 cells/ mm3, with a range of 271 to 950 cells/ mm3. 34.00% of the patients had counts which were sub-normal (less than 500 cells/ mm3), and 66.00% of the patients had normal counts. The mean CD4+ count in 32 patients of TBM was 481.75 cells/ mm3, with a range of 270 to 670 cells/ mm3. Significantly, in the TBM group, 53.12% of the patients had CD4+ counts which were sub-normal. Only 46.88% of the patients had normal CD4+ counts. On comparing the CD4+ counts in the two groups of patients using the unpaired student t-test, a significant difference was attained (p value 0.031), which lead to the conclusion that the CD4+ counts in patients of TBM were significantly lower than the patients of PTB.

Next the comparisons of BMI and CD4+ counts were done to find the correlation between under-nutrition and T-lymphocyte counts. In the PTB group of 50 patients, 6 patients were undernourished according to BMI, of which 5 had CD4+ counts less than 500 cells/ mm^3. On the contrary, of the 19 overweight patients, 17 had normal CD4+ counts. The mean CD4+ count in the underweight PTB patients was 366.40 cells/ mm^3. In stark comparison, the mean CD4+ count in the overweight PTB patients was 641.32 cells/ mm^3. An increase in BMI lead to a progressive increase in CD4+ counts. In the TBM group of 32 patients, 8 patients were undernourished according to BMI, out of which 7 had CD4+ counts of less than 500 cells/ mm^3. On the contrary, of the 7 overweight patients, 6 had normal CD4+ counts. The mean CD4+ count in the underweight TBM patients was 404.38 cells/ mm^3. In stark comparison, the mean CD4+ count in the overweight TBM patients was 557.38 cells/ mm3. An increase in BMI lead to a progressive increase in CD4+ counts. Hence the results of the current study concluded that an increase in BMI in both sets of patients was associated with a significant increase in CD4+ counts.

Next, the relationship between the grading of sputum positivity and CD4+ counts was established. A previous study (13) had proven that a higher grading of sputum positivity of AFB in PTB was associated with a worse prognosis.. In this study the mean CD4+ count of cases with 3+ sputum (412.5 cells/ mm^3) was significantly lower than 1+sputum positive (599.0 cells/ mm^3) with a p value of 0.037.

Hence, the study concludes that CD4+ counts were significantly lower in TBM patients as compared to PTB patients. Both sets of patients had counts lower than the accepted lower limits of normal values despite the absence of HIV. Furthermore, the CD4+ counts in both sets of patients progressively increased with an increase in the BMI. The major limitation of the current study is inability to appreciate whether the CD4+ counts will improve on completion of effective anti-tubercular therapy. Furthermore we are unable to assess whether the lower CD4+ counts predispose to extra-pulmonary TB or the extra-pulmonary TB per se reduces the CD4+ counts. Also, a small sample size and a cross sectional nature of the study was a limitation. The study strongly emphasizes the importance of improving the nutritional status of the masses in a developing nation like ours, as it not only reduces the morbidity and mortality associated with TB, but also improves the immune status and the CD4+ counts.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced it's outcome. ,

Table 1. Age-wise distribution of patients with PTB and TBM

Serial No.	Age Group	PTB (n=50)		TBM (n=32)	
		No. of Patients	%	No. of Patients	%
1.	10 – 20	7	14.00%	5	15.63%
2.	21 – 30	9	18.00%	6	18.75%
3.	31 – 40	8	16.00%	3	9.38%
4.	41 – 50	6	12.00%	8	25.00%
5.	51 – 60	2	4.00%	4	12.50%
6.	61 – 70	10	20.00%	4	12.50%
7.	> 70	8	16.00%	2	6.25%

Mean age of patients was 45.88±22.81 years for PTB (with an age range of 11 to 88 years), and 42.43±18.38 years (with an age range of 14 to 76 years) for TBM.

Table 2. Sex-wise distribution of patients with PTB and TBM

Sex	PTB (n=50)		TBM (n=32)	
	No. of Patients	%	No. of Patients	%
Male	27	54.00%	19	59.38%
Female	23	46.00%	13	40.63%

Males outnumbered females in both sets of patients.

Table 3. BMI of patients with PTB and TBM

BMI	PTB (n=50)		TBM (n=32)	
	No. of Patients	%	No. of Patients	%
< 18.5	6	12.00%	8	25.00%
18.5 to 20.99	15	30.00%	10	31.25%
21 to 23.5	10	20.00%	7	21.87%
> 23.5	19	38.00%	7	21.87%

Mean BMI in PTB group was 22.95±5.20 kg/ m^2 in PTB and 21.81±4.97 kg/m^2 in TBM group (p – 0.325).

Table 4. CD4+ counts in patients with PTB and TBM

Cd4+ counts (cells/ mm^3)	PTB (n=50)		TBM (n=32)	
	No. of Patients	%	No. of Patients	%
< 300	2	4.00%	1	3.13%
300 – 400	7	14.00%	6	18.75%
401 – 500	8	16.00%	10	31.25%
501 – 600	11	22.00%	9	28.12%
601 – 700	16	32.00%	6	18.75%
> 700	6	12.00%	0	0

Mean CD4+ count is PTB was 541.54±130.61 cells/ mm3 and in TBM was 481.75±102.11 cells/ mm3 with a 'p' value of 0.031

Table 5. Mean CD4+ counts with relation to BMI in PTB patients

BMI (kg/ m2)	No of PTB cases	Mean CD4+ count (cells/ mm^3)	± SD
< 18.5	6	366.40	78.08
18.5-20.99	15	477.87	106.31
21-23.5	10	576.80	104.66
> 23.5	19	641.32	115.44
Total	50	541.54	130.61

In patients of PTB, the mean CD4+ counts progressively increased with increase in BMI.

Table 6. Mean CD4+ counts with relation to BMI in TBM patients

BMI (kg/ m2)	No. of TBM cases	Mean CD4+ count (cells/ mm^3)	± SD
< 18.5	8	404.38	74.56
18.5-20.99	10	449.90	70.90
21-23.5	6	537.17	133.38
> 23.5	8	557.38	63.18
Total	32	481.75	102.11

In patients of TBM, the mean CD4+ counts progressively increased with increase in BMI.

Table 7. Relationship of sputum positivity with mean CD4+ counts

Sputum for AFB	No of PTB patients	Mean CD4+ count (cells/ mm^3)	± SD
1+	12	599.0	89.2
2+	16	557.5	128.4
3+	22	412.5	162.6
Total	50	523.4	140.9

The mean CD4+ count of cases with 3+ sputum was significantly lower than 1+ sputum positive (p – 0.037).

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