

Influence of Body Mass Index and Anti-Tubercular Therapy on the Lymphocyte Profile in Elderly Tuberculosis Patients

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Abstract

Elderly individuals are at risk of infection with *Mycobacterium tuberculosis* due to immunosenescence, nutritional deficiency and fall in standard of living. Decline in number and function of some key cells in the immune system have been documented in patients with tuberculosis, although inadequately. In a prospective longitudinal study, thirty elderly patients with pulmonary tuberculosis and an equal number of age and sex matched controls without tuberculosis were investigated. The mean CD4+ and CD8+ lymphocyte and NK cell counts among patients at diagnosis were lower than controls ($p < 0.001$). The cases had lower body mass index (BMI), serum protein and serum albumin levels, indicating an inferior nutritional status compared to controls. The mean CD4+, CD8+ lymphocytes and NK cell counts significantly correlated with each other and with total lymphocyte count, extent of disease as established by chest x-ray and nutritional status. Multivariate stepwise linear regression analysis showed that BMI correlated best with the observed cell counts. Following treatment, the cell counts recovered but continued to be lower than healthy controls.

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Introduction

Elderly individuals of all races and ethnicity are at a very high risk of infection with *Mycobacterium tuberculosis*.¹⁻³ India has the highest number of tuberculosis cases in the world with two million new cases and five hundred thousand deaths each year.⁴ Consequently tuberculosis is a major problem in older people and has emerged as the fifth leading cause of death in these individuals.⁵ The risk appears to be higher in older people who live in nursing homes and long term care institutions.^{6,7} Senescence has a significant impact on cell-mediated immunity rather than humoral immunity.^{8,9} Protective immunity for tuberculosis is primarily a function of cell-mediated immunity. Senescence can be logically expected to predispose a person to tuberculosis. In addition,

secondary immunodeficiency due to nutritional deficiency and drugs can increase the risk of tuberculosis in this age group.¹⁰

Tuberculosis in older patients can be a result of reactivation of primary infection that may be in a latent state.¹¹ The precise reason for this vulnerability to tuberculosis remains uncertain which may be reactivation of latent tuberculosis or the loss of acquired immunity against the organism in older people.

We evaluated older patients with tuberculosis to establish a relationship between T cell subset and NK cell counts, the extent of clinical disease, nutritional status, and the influence of anti tubercular therapy on the lymphocyte profile.

Material and Methods

Sixty individuals aged 60 years and above, free of other systemic illness and HIV sero negative were investigated. Thirty elderly individuals who had pulmonary tuberculosis constituted the cases and the rest thirty age and sex matched subjects with no tuberculosis constituted the control group. All cases and controls belonged to the geographic area of National Capital Region of Delhi, attending the Geriatric Out Patient

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Services of the AIIMS Hospital. None of the cases or controls had been exposed to BCG vaccination in their childhood (universal immunization program started in India in 1960s). Pulmonary tuberculosis in cases was diagnosed based on the criteria of compatible clinical and radiological features and detection of acid fast bacilli in sputum.

Following recruitment, the patients and controls were assessed for their nutritional status by estimation of BMI, serum protein, serum albumin and quantification of the extent of disease on a chest x-ray as per the recommendations of National Tuberculosis Association of USA. In view of the high endemicity of tuberculosis in India, no attempt was made to assess the PPD status of the controls. For similar reasons, no attempt was made to define the infection as primary or reactivation in the cases included in the study.

Peripheral blood mononuclear cells were stained with monoclonal antibodies (Becton Dickinson CD4/CD8 Simultest, CD3/CD56 Simultest). The stained cells were analyzed flowcytometrically (Becton Dickinson, La Jolla, CA, USA) using the CellQuest software. For each sample 10,000 events were collected and analyzed by flowcytometry. A simultaneous complete blood count and absolute lymphocyte count were determined. The absolute number of CD4+, CD8+ and NK cell counts was determined by multiplying the respective percentages derived from FACS gating with the absolute lymphocyte number per dl of each population.

Patients were treated with short course chemotherapy regimen 2HRZE/4HR {two months of

isoniazid (5mg/kg), rifampin (10mg/kg), pyrazinamide (15-30 mg/kg) and ethambutol (15-25 mg/kg); and four months of isoniazid (5mg/kg) and rifampin (10mg/kg)}. Lymphocyte subset analysis was repeated after two months of therapy.

Cases and controls were classified into three groups: cases before treatment, cases after treatment and controls. The data was analyzed using the software SPSS 9.0 for Windows. Quantitative variables were compared using non parametric statistical test namely Kruskal-Wallis test. Correlation between the variables was determined by using Pearson's correlation analysis. Multivariate stepwise linear regression analysis was used to identify independent predictors of low T cell subsets and NK cell counts. All p values less than 0.05 were considered as significant.

Results

The mean age of cases and controls was 65.8 years (range 60 to 96 years). Patients were graded for severity of tuberculosis by chest x-ray features as per the recommendations of National Tuberculosis Association of USA. Minimal disease was seen in 12 patients and moderate to advanced form of the disease was seen in the remaining 18 patients (Table 1). The total leukocyte count and total neutrophil count were significantly higher in cases compared to controls ($p < 0.001$) whereas the total lymphocyte count in cases and controls did not differ. However, the mean CD4+ lymphocyte, CD8+ lymphocyte and NK cell count among patients at the time of recruitment was significantly lower than controls ($p < 0.001$, Table 2). Similarly the nutritional indicators (BMI, serum protein

Table 1. Severity of disease in chest x-ray of cases

Severity of disease	No. of cases
<i>Minimal disease</i>	
No cavitation(s) / Total extent does not exceed volume of lung above second chondrosternal junction.	12
<i>Moderately advanced</i>	8
Disseminated lesions of moderate density extending to total volume of one lung or equivalent in both lungs or dense lesions limited to one third of one lung or total diameter of cavitation < 4cm	
<i>Far advanced</i>	10
More extensive than moderately advanced disease	

Table 2 . Mean leukocyte, neutrophil, lymphocyte and lymphocyte subset count (\pm standard deviation) in cases before treatment and healthy controls

Cell types	Cases (n=30)	Controls (n=30)	P value
Total leukocyte	11023 \pm 2322	7387 \pm 1629	< 0.001
Neutrophil	8603 \pm 1825	4814 \pm 1212	< 0.001
Lymphocyte	2372 \pm 408	2488 \pm 378	not significant
CD 4+ lymphocyte	423 \pm 246	687 \pm 154	< 0.001
CD8+ lymphocyte	409 \pm 290	670 \pm 155	< 0.001
NK cell	211 \pm 176	428 \pm 190	< 0.001

and albumin) were significantly deficient in patients compared to controls. The mean BMI in cases was $15.23 \pm 2.56 \text{ kg/m}^2$, which was significantly lower than $24.06 \pm 2.07 \text{ kg/m}^2$ in controls ($p < 0.001$). The mean serum protein and serum albumin levels in the cases were $6.52 \pm 0.81 \text{ gm/dl}$ and $3 \pm 0.54 \text{ gm/dl}$ and were significantly lower than the levels of $7.66 \pm 0.37 \text{ gm/dl}$ and $4.22 \pm 0.26 \text{ gm/dl}$ respectively in controls ($p < 0.001$).

Correlation between different variables was determined using Pearson's correlation coefficient. In the disease group, CD4+ lymphocyte count was most significantly correlated with CD8+ lymphocyte count ($p < 0.001$). Other factors which showed significant correlation with CD4+ lymphocyte count were BMI ($p < 0.005$), total lymphocyte count ($p < 0.005$), NK cell count ($p < 0.005$) and the extent of disease on chest x-ray ($p < 0.01$). There was significant correlation ($p < 0.001$) of CD8+ lymphocyte count with total lymphocyte count, CD4+ lymphocyte count and NK cell count. CD8+ lymphocyte also showed significant correlation with the extent of disease on chest radiograph ($p < 0.05$), serum protein level ($p < 0.005$) and BMI ($p < 0.05$). NK cell count was strongly correlated with total lymphocyte count ($p < 0.001$) and CD8+ lymphocyte count ($p < 0.001$). Other variables that significantly correlated with NK cell count were CD4+ lymphocyte count ($p < 0.005$), BMI ($p < 0.05$) and the extent of disease on chest radiograph ($p < 0.05$).

To identify the independent predictors of low CD4+ lymphocyte count, multivariate stepwise linear regression analysis was carried out. The variables included in the regression equation were the extent of disease on chest x-ray, serum protein concentration, serum albumin concentration, total leukocyte count, total lymphocyte count, CD8+ lymphocyte count and NK cell count. The total CD8+ lymphocyte count was the best single predictor of CD4+ lymphocyte count ($r = 0.616$, $p < 0.001$). CD8+ lymphocyte alone could predict 38% of the

change in CD4+ lymphocyte count. This predictive value was increased significantly by adding BMI ($r = 0.688$, $p < 0.05$), the value being increased to 47%. No other variable added significantly to this predictive value. If cases and controls were taken together and analyzed, it was found that total lymphocyte count also increased the predictive accuracy ($r = 0.728$, $p < 0.001$). These three variables could explain 53% of change in CD4+ lymphocyte count.

Using the identical methods, independent predictors of NK cell count were identified. BMI emerged as the single best predictor of low NK cell counts ($r = 0.55$, $p < 0.001$), with low BMI being directly correlated to low NK cell count. The predictive value of this analysis was increased by 5% if total lymphocyte count was added in the analysis, with no other variable adding significantly to its predictive value.

Stepwise linear regression analysis was performed to determine clinical correlates of lymphocyte subset count, using CD4+ lymphocyte as the dependent variable and age, bacillary load (degree of sputum positivity), extent of disease on chest radiograph, serum total protein levels, serum albumin levels and BMI as independent variables. Total leukocyte count, total lymphocyte count, CD8+ lymphocyte count and NK cell count were excluded from the analysis. Here it was found out that only BMI could reliably predict the change in CD4+ lymphocyte count ($r = 0.54$, $p < 0.002$), predicting 29% of the change. Using the same method with NK cells as dependent variable, again BMI emerged as the single best predictor of change ($r = 0.42$, $p < 0.02$). No other variable added significantly to the predictive value of BMI.

Two patients succumbed to the disease early in the course and two patients could not be contacted for follow up investigation. Twenty six patients were included in the comparison in post intensive therapy analysis. An improvement in subset counts was seen

Table 3. Mean lymphocyte count (\pm standard deviation) in cases before and two months after treatment

Cell type	Before treatment (n=26)	After treatment (n=26)	P value
CD 4+ lymphocyte	445 \pm 236	641 \pm 284	< 0.001
CD8+ lymphocyte	454 \pm 283	546 \pm 272	< 0.005
NK cell	235 \pm 176	310 \pm 186	< 0.001

in the cases after two months of anti-tubercular therapy, which was statistically significant for all three subsets evaluated (Table 3). The recovery in CD4+ lymphocyte and NK cell series was nearly complete as the mean counts did not differ from the values among controls whereas the CD8+ lymphocyte count continued to be significantly lower than healthy controls ($p < 0.05$).

Discussion

We observed that CD4+ lymphocyte, CD8+ lymphocyte and NK cell count were below normal in most of the patients with tuberculosis. The CD4+ lymphocyte count in some cases were as low as that seen in HIV infected individuals. In contrast to HIV infected patients in which CD4+ lymphocyte count falls independently of the CD8+ lymphocyte count, our study demonstrated that in tuberculosis patients, there is a parallel fall in CD4+ lymphocyte, CD8+ lymphocyte and NK cell counts as well as the total lymphocyte count. The extent of decline of CD4+ lymphocytes correlated strongly with low CD8+ lymphocyte count. Other variables showing significant correlation were BMI, total lymphocyte count, NK cell count and the extent of disease on chest x-ray. Linear stepwise discriminant analysis revealed that only CD8+ lymphocyte count and BMI were the independent predictors of CD4+ lymphocyte count and no other variable added to their predictive value. Together these could account for a 47% change in CD4+ lymphocyte count. This also indicates that there are other variables still unknown to us, which may affect CD4+ lymphocyte count. The NK cells showed a similar decline in count in tuberculosis patients. This decrease strongly correlated with total lymphocyte count and CD8+ lymphocyte count. Other variables found to be significantly correlated with NK cell count were CD4+ lymphocyte count, BMI and the extent of disease on chest x-ray. On linear stepwise discriminant analysis BMI emerged as the single best predictor of NK cell count, low BMI being directly correlated to low NK cells. The predictive value of this analysis was increased on adding total lymphocyte count to the variables, no other variable adding significantly to its predictive value. The change in these lymphocyte subsets in tuberculosis improved with anti-tubercular therapy in most patients in our study. This improvement

was documented in numbers of both CD4+ lymphocyte and NK cell.

Such improvement in CD4+ lymphocyte numbers with anti-tubercular therapy has been documented in the literature.^{2,12,13} But the decrease in NK cells in tuberculosis as well as their rise after anti-tubercular therapy has not been previously reported. Nirmala et al reported decreased NK cell activity but not number or proportion of NK cells in a group of tuberculosis patients who were aged less than sixty years of age.¹⁴ The reduced NK cell activity was considered as the effect of tuberculosis infection rather than the cause of the disease. Although the improvement in lymphocyte subset count is definitely related to the effect of treatment, some contributions from general improvement in health as a result of control of infection cannot be excluded.

Patients with tuberculosis have been documented to have decreased production of interferon- γ .¹⁵ Experimental and clinical tuberculosis has been reported to be associated with normal, depressed or elevated number of various lymphocyte subsets.^{12,16-21} The effects of *Mycobacterium tuberculosis* on the compromised immune status of older subjects has not been assessed. The protection against tuberculosis is predominantly provided by the CD4+ T cells, CD8+ T cells playing a complementary role with the help of innate immunity from various mononuclear cells and NK cells.^{22,23} In the present study, all the three cell types were depressed with associated poor nutritional status as indicated by low BMI which was an important determinant of this phenomenon. The adverse impact of malnutrition on both arms of the immune system especially in the extremes of life has been documented.²⁴ We suspect that in older patients with sub-clinical malnutrition, reactivation of latent tuberculosis or re-infection is facilitated by a decline in the cell-mediated immunity. Tuberculosis in turn leads to further decline in food intake (due to anorexia) and subsequently further fall in immune status. This vicious cycle may explain the observation that moderate to severe malnutrition not only predisposes to tuberculosis but also is a risk factor for early death.²⁵ Treatment of

tuberculosis breaks this cycle and restores the immune status and nutritional status.

The influence of nutritional factors, smoking, and alcoholism has been reported as factors affecting lymphocyte population. Alcoholism and smoking affect the CD8+ numbers, whereas NK cells were affected by smoking.¹⁶ Besides, the influence of pathogenic mycobacteria on circulating levels of immunocompetent cells and other mediators of immunoprotection have not been understood. Thus, it would be useful to investigate direct effects of mycobacterial derived products on the competence of immune system in handling pathogenic mycobacteria in older people compared to younger people.

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