Eur J Med Res (2011) 16: 285-288

CD4+ T-Lymphopenia in HIV Negative Tuberculous Patients at King Khalid University Hospital in Riyadh, Saudi Arabia

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Abstract
Tuberculosis (Tb) is a chronic infectious disease in which the cellular immunity (specifically CD4+ and CD8 lymphocytes) provides the most important defense in controlling infection. CD4 lymphopenia is a well-defined risk factor for the development of active tuberculosis in patients infected with Human Immunodeficiency Virus. In HIV - negative patients, CD4 and CD8 cell count suppression has been associated with Tb infection. Our study was designed to determine the baseline and post-treatment values of CD4 and CD8 in HIV negative patients diagnosed with active Tb in Saudi Arabian patients. We recruited twenty-eight, non-HIV patients with tuberculosis for the study group comprising 16 males and 12 females with either disseminated or localized active Tb infection. Two control groups were selected – one of twenty-one matched healthy controls and the second of forty-two subjects from pool of controls of an ongoing study in same population for normal CD4 and CD8 counts. The baseline pre-treatment CD4 and CD8 counts in the study group were significantly lower than either control group. Specifically the mean ± SD of CD4 counts were 556.79 ± 298.81 in the study group vs 1,132.38 ± 259.90 in control group 1 and 1,424.38 ± 870.98 in control group 2 (p 0.000). Likewise the CD8 counts in the study group were 1,136.00 ± 512.06 vs. 1,461.90 ± 367.02 in control group 1 and 1,495.90 ± 565.32 in control group 2 (p 0.000) respectively. After treatment of tuberculosis, the study patients experienced a significant increase in their mean ± SD CD4 and CD8 cell counts, from 556.79 ± 297.81 to 954.29 ±210.90 for CD4 cells (p 0.005) and 1136.00 ± 512.06 to 1,316.54 ± 286.17 for CD8 cells (p 0.002). Analysis of study patients with disseminated disease found significantly lower CD4 cells (but not lower CD8 cells) compared to study patients with localized disease, both at baseline and after treatment. The mean ± SD baseline CD4 cells were 247.60±187.80 with disseminated vs 728.56 ± 186.32 for localized disease (p = 0.000) which rose to 842.30 ± 93.55 vs 1016.50 ± 233.51 (p = 0.033) respectively. We conclude that tuberculosis may be associated with CD4 and CD8 lymphopenia even in patients without human immunodeficiency virus infection, there was the tendency of recovery towards normality especially of the CD4 and CD8 counts after treatment, and that disseminated disease is associated specifically with profound CD4 lymphopenia.

Introduction

Tuberculosis remains an infectious disease causing significant morbidity and mortality on a global scale. The fifteenth annual report of the World Health Organization [1] gave an estimate of the global burden of the disease caused by TB in 2009 as follows: 9.4 million new cases, prevalent cases of 14 million and deaths of 1.3 million and 0.38 million among HIV-negative and HIV positive people respectively.

The natural course of TB infection is basically determined by the ability of the host's immune system resulting in swift eradication, dormancy or failure, which leads to active disease [2]. Protective immunity to TB in humans relies upon both CD4+ and CD8+ T-cells through cell-mediated responses, allowing full eradication or control of infection [3]. The important contribution of CD4+ T- cells is especially obvious in HIV infection which by depletion of these subsets of cells predisposes infected individuals to reactivation of tuberculosis. It had previously been documented that Tb infection itself may cause a CD4 lymphopenia in patients not infected with HIV, sometimes with grave consequences [4]. It had also been demonstrated that the CD4 lymphoenia was potentially reversible with treatment [5]. The aim of this study was to determine CD4 and CD8 values of non-HIV tuberculosis patients at baseline and post treatment periods by flow cytometry.

Material and Methods

We enrolled a study population of twenty-eight randomly selected non- HIV infected patients with wide spectrum of active tuberculosis seen at the King Khalid University Hospital (KKUH), Riyadh over a one-year period. Eligible patients were enrolled based on compatible symptoms of TB and positive Mycobacterium tuberculosis based on Ziel -Nielsen smear and/or culture of relevant specimens as determined by the Bactec system and/ or Lowenstein-Jensen culture methods. The first control group of matched

healthy controls was not infected with Tb confirmed by absence of history suggestive of tuberculosis, negative tuberculin tests and normal chest x-rays. The second control group was derived from an ongoing study determining the normal range of CD4 and CD8 counts from healthy controls of the same population at KKUH. For this second control group, Quantiferon negativity was additionally used to exclude latent tuberculosis. Both subjects and controls were screened for HIV and were ensured negative using Enzyme-linked immunosorbent assay (ELISA) and Recombinant immunoblot assay (RIBA). Patients and controls were excluded if they have the following conditions: any form of immunodeficiency syndromes, diabetes mellitus, chronic kidney disease and concurrent use of immunosuppressant medications. Informed consent was sought from both subjects and controls before enrollment. Baseline and post- treatment CD4/CD8 counts in recruited patients and baseline parameters of controls were recorded. Patients in the study group were treated for two months with isoniazid, rifampicin, pyrazinamide and ethambutol. The former two drugs were then continued to complete treatment of 6, 9, and 12 months for pulmonary, adenitis, and military/disseminated/meningitis respectively. Cure was determined by such parameters including resolution of fever, increase in weight, radiological resolution and decrease in erythrocytes sedimentation rate.

Flow Cytometry Study

Blood was collected in EDTA tubes and analyzed within 6 hours of storage for direct immunoflourescence stain. Twenty (20) ul of fluorochromes conjugated monoclonal antibody was added to 100µl of whole blood in Falcon tube. The sample was then spun gently and incubated for 15 minutes in dark at a room temperature. The sample was further left for 10 minutes after adding 2 ml of FACS lysing solution. Centrifugation was done at 500 x g for 5 minutes followed by discarding of the supernatant. The cells pellet was then re-suspended in 2 cc of wash buffer. Further centrifugation was done and cells were re-suspended in 0.5cc of paraformaldehyde for flow cytometric analysis. Acquisition was performed on FAC Scan (Becton Dickinson Immunocytometry System). CELL Quest Software BDIS on list mode data was then utilized to determine the lymphocyte gate by forward / side scatter characteristic. FLI/FL2 contour plots were finally employed for two-color analysis.

Recorded data was analyzed using SPSS version 16 Statistical package. Significance was determined at p value of 0.05.

Results

Twenty-eight patients were recruited for the study group, made up of 16 males and 12 females with a combined mean age of 40 (Range 17-69) years. Table 1 shows the composite data of their total white blood cells with mean+ SD of 9,653.6 ± 5,602.3 (95% confidence interval 7,436.5-11,900.4), lymphocytes 3,753.7 ± 304.4 (2,549.4-4,958.0) and percentage lymphocytes 36.2 ± 15.2 (30.2-42.2) of the study patients. These values were within normal reference values in our laboratory. Baseline mean±SD CD4 counts of study patients (Table 2) were significantly lower at 556.79 ± 297.81 versus 1132.38 ± 259.90 in control group 1 and 1424.38 ± 870.98 in control group 2 (pvalue 0.000). Likewise, CD8 counts of study patients were significantly lower as at 1136.00± 512.06 compared with either of control groups at 1461.90 ± 367.02 and 1495.90 ± 565.32 respectively (p value of 0.000). After treatment, the study group experienced significant improvement in CD4 and CD8 counts. The mean± SD count of CD4 cells went up from 556.79 ± 297.81 pretreatment up to 954.29 ± 210.90 after (p 0.005), while the CD8 cells were 1136.00± 512.06 and 1316.54 ± 286.17 respectively (p 0.002). Patients with disseminated disease (miliary and localization in more than one tissue) showed significantly lower initial CD4 counts (Table 3) than localized form (Pulmonary or Adenitis), with mean ± SD CD4 counts of 247.60 ± 187.80 versus 728.56 ± 186.32 (p value 0.000). No significant dif-

Table 1. Composite Data of Total White Blood Cells (WBC), Lymphocytes and Percentage Lymphocytes among 28 patients of the Study Group.

Parameter	Total WBC	Lymphocytes	Percentage of Lymphocytes
Mean	9,652.6	3,753.7	36.2
Standard Deviation	5,602.3	304.4	15.2
95% Confidence Interval	7,436.5-11,900.4	2,549.4-4,958.0	30.2-42.2

Table 2. Comparison of Pretreatment versus Controls and Post-treatment in CD4 and CD8 Counts

CD4 Counts	mean ± SD	p Value	CD8 Counts	mean ± SD	p Value
Pretreatment	556.79 ± 297.81	0.000	Pretreatment	1136.00 ± 512.06	0.000
Controls 1	1132.38 ± 259.90		Controls 1	1461.90 ± 367.02	
Controls 2	1424.38 ± 870.98	0.000	Controls 2	1495.90 ± 565.32	0.000
Pretreatment	556.79 ± 297.81	0.005	Pretreatment	1136.00 ± 512.06	0.002
Post-treatment	954.29 ± 210.90		Post-treatment	1316.54 ± 286.17	

Table 3. Comparison of Pretreatment and Post-treatment CD4 and CD8 Counts between Disseminated and Localized Diseases.

Pretreatment CD4 Counts	**mean ±SD**	**p Value**	**Pretreatment CD8 Counts**	**mean ±SD**	**p Value**
Disseminated	247.60±187.80	0.000	Disseminated	994.70±549.12	0.285
Localized	728.56±186.32		Localized	1188.61±388.39	
Post-treatment CD4 Counts			**Post-treatment CD8 Counts**		
Disseminated	842.30±93.55	0.033	Disseminated	1368.90±307.35	0.481
Localized	1016.50±233.51		Localized	1287.44±278.47	

ferences in CD8 counts were found between patients with disseminated and localized forms of the disease. The post treatment value of CD4 counts in the disseminated disease remained lower than localized disease even after treatment at 842.30 ± 93.55 versus 1016.50 ± 233.51 ($p = 0.033$).

Discussion

Humans acquire infection with Mycobacterium tuberculosis (MTB) commonly by inhaling the bacterium. Only a small number of bacilli need to enter distal alveoli of the human's lung to establish infection [6]. The alveolar macrophages form first line of defense against the inhaled droplets containing MTB [7]. Cellular-mediated immunity has a central role in the containment of TB. The mechanism involves rapid onset of Th1 cytokine response comprising interferon (IFN) γ [8] and tumour necrosis factor (TNF)-α [9]. Furthermore, restricted mycobacterial growth in the lungs had recently been proposed through the induction of Th17 cells leading to production of IL-17 and IL-23. These cytokines cause inflammation and recruitment of Th1 cytokine producing cells [10, 11]. CD8+ T-cells had also been shown to be essential in effective T-cell immune response [12].

The majority of patients successfully contain the primary infection within 2-10 weeks and go on to develop a vigorous delayed-type hypersensitivity (DTH) response [2]. In most healthy adults, adaptive immunity mediated by T- cells controls (but does not eradicate) Tb infection [13]. Thus ongoing protective immunity is required to maintain control over the bacilli. Immune failure of the adaptive immunity results in clinical tuberculosis [14]. The HIV pandemic provides direct evidence that loss of CD4 T-cell numbers and functions resulted in progressive primary infection, reactivation of endogenous Tb and enhanced susceptibility to re-infection [15].

Many studies had documented low CD4 counts in patients with tuberculous diseases, with few of them showing the reversibility of the counts towards normality after treatment. Similar to our study, Uppal et al [5] and Davoudi et al [16] found CD4 lymphopenia in patients with tuberculosis. Singhal and Banavalikar [17] also found low CD4 counts in both smear positive and smear negative patients with pulmonary tu-berculosis. In addition Kony et al [18] in a study in Senegal found extra-pulmonary and more especially miliary to have substantially lower CD4 counts. This concurs with our study which demonstrated significantly lower CD4 counts in disseminated than localized disease. Other studies had inferred that patients with low CD4 counts tend to have severe disease [19, 20] with profound incapacity.

Many hypotheses had been brought forward to explain the depletion of CD4+ which include unusual response to infections and homing of lymphocytes to affected tissue than in circulation [12]. Similar to our study, HIV- negative patients with TB and CD4+ T-lymphocyte depletion on presentation were shown to normalize their CD4+ cell counts with TB treatment [21]. Similarly, Bose et al [22] studied 21 cases of chronic, multi-bacillary pulmonary TB refractory to treatment and found that the decreased mean CD4/CD8 ratio normalized only in those patients who responded clinically and bacteriologically to therapy; in refractory cases, the ratio remained low.

Reports on CD8 counts in HIV-negative patients with TB had been conflicting. Shijubo et al [23] found significantly decreased CD8 cells, which is similar to our finding of significantly lower CD8 in patients as compared with controls. Davoudi et al [16] found lower number of CD8 cells in patients with severe disease like meningitis/miliary forms of TB as compared with controls. The reversibility of CD4 lymphopenia with successful therapy as found in our study was also demonstrated in various studies [5, 21, 22].

In conclusion, our study found significantly lower CD4 and CD8 counts among Tb infected HIV negative patients as compared with controls. The post-treatment CD4 counts demonstrated the tendency towards recovery to normal values. Patients with disseminated disease had much lower CD4 values than localized forms, with a delay of returning towards normality.

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Received: January 6, 2011 / Accepted: March 16, 2011

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