PRELIMINARY STUDY ON CIRCULATING SERUM LEVELS OF TUMOR NECROSIS FACTOR – ALPHA IN PATIENTS OF TUBERCULOSIS

Ritesh Gupta, H. Krishna Prasad*, Kalpana M. Nagarkar and A. B. Dey.

Department of Medicine and Department of Biotechnology*, All India Institute of Medical Sciences, New Delhi – 110029.

ABSTRACT

Tumor necrosis factor -alpha (TNF- α) has been implicated in the pathogenesis of several non-infectious and infectious diseases including tuberculosis. In a prospective longitudinal study, TNF- α level in blood was estimated by sandwich ELISA using anti human TNF- α antibody, in 22 patients with active pleuro-pulmonary and lymphnode tuberculosis before and after chemotherapy and 8 healthy controls. Six patients and six controls had detectable levels (> 5 pg/ml) of TNF- α in blood. The mean TNF- α levels in controls and cases before and after treatment were 182.4pg/ml, 896.7 pg/ml and 678.7pg/ml pg/ml respectively. Though not statistically significant, there was a trend towards younger age, shorter duration of symptoms, presence of fever and anorexia, and high ESR, in patient with high serum TNF- α levels.

KEY WORDS: Tumor necrosis factor, cytokines, tuberculosis.

INTRODUCTION

Tumor necrosis factor alpha (TNF- α) is an important cytokine in the pathogenesis of several infectious and non-infectious diseases; and a variety of malignancies (1). A number of cytokines including TNF- α is released following activation of T-lymphocytes and tissue macrophages in the pathogenesis of tuberculosis (2,3). TNF- α has broad range of biological actions with beneficial effect in eliminating *M. tuberculosis* (3). These protective effects are also accompanied by cytotoxic effects namely; tissue necrosis and cavity formation locally; and weight loss, anorexia and fever systemically (4). Circulating levels of TNF- α were measured in the present study to ascertain any association with clinical manifestations of the tuberculosis in different forms.

MATERIALS AND METHODS

Newly diagnosed and untreated patients with pleuro-pulmonary and lymph node tuberculosis were included in this study. The diagnosis of tuberculosis was made after demonstration of the organism or compatible histopathological picture. Eight healthy subjects were also included as controls after excluding tuberculosis on the basis of clinical and radiological features. Five ml blood was collected from cases and controls. Serum was separated and was stored at -20°C till TNF- α estimation. Patients were treated with standard short course chemotherapy comprising of isoniazid, rifampicin, ethambutol and pyrazinamide in single daily dose calculated on the basis of body weight. After 2 months of intensive therapy blood samples were again collected and sera stored for TNF- α estimation.

Estimation of TNF-alpha

Author for correspondence :

Dr. A. B. Dey, at the above address.

TNF- α was estimated by a sandwich type enzyme

linked immunosorbent assay (ELISA) using anti human TNF- α antibody (Endogen, USA). Assay was performed according to the manufacturer's instructions. 50 μ l of TNF- α standard and samples were added to each well along with 50 μ l of biotinylated antibody reagent. The plates were incubated at 25°C for 2 hrs. After washing the plates, streptavidin horseradish peroxidase solution was added and the plates were further incubated for 25°C for 30 mins. The plates were washed. The substrate was added and the plate was developed in dark for 30 mins. The reaction was terminated and the plate was read using an ELISA reader (Molecular Devices, USA) at 490nm. Each sample was run in triplicate. Values differing more than 10% of the mean of three values were discarded. A standard curve was drawn. Amount of TNF- α in each sample was determined by interpolating from the absorbance value (y-axis) to the TNF- α concentration (x-axis) using the standard curve. The lower limit of detection of TNF- α by this assay was 5pg/ml.

RESULTS AND DISCUSSION

Out of 29 patients recruited to the study, seven were lost to follow up and excluded from analysis. Twenty two patients (M:F = 12:10) and 8 healthy volunteers as controls (M:F = 5:3) are subjects of this communication. The mean age of cases and controls were 31.7 ± 10.8 years and 32.5 ± 11.9 years respectively. Fourteen patients had pulmonary tuberculosis (6 cavitary disease and 8 non-cavitary disease in chest x-ray), five had lymphnode tuberculosis and three patients had tuberculous pleural effusion. The mean duration of symptoms in patients was 147.5 days ranging between 15 days and 2 years. Fever and cough were the most frequent symptoms followed, in order, by expectoration, anorexia, weight loss, chest pain, lymphadenopathy, hemoptysis and dyspnoea. The signs encountered were consistent with the clinical diagnosis. Tuberculin positivity (10mm x 10mm induration) was detected

in 11 patients.

TNF- α levels ranged between 7pg/ml to 750 pg/ml (mean 182.4, SEM 122.9) in six of the eight controls in whom it was detectable. Before treatment, 16 patients had undetectable serum levels of TNF- α and the rest had levels between 5pg/ml and 2500 pg/ml (mean 896.7, SEM 431.6) with very high values in 3 patients. There was no significant difference in age, sex, clinical features and laboratory manifestations among patients with very high TNF- α values and those with very low or undetectable levels. However, there was a trend towards younger age, shorter duration of symptoms, presence of high fever, presence of loss of appetite and higher ESR. There was no statistical difference between the serum level of TNF- α of cases and controls. Following intensive chemotherapy for 2 months serum TNF- α levels were detectable in 6 patients ranging between 5 pg/ml and 1850 pg/ml; mean 678.7, SEM 324.4. Decline, though not statistically significant, was observed in all patients who had detectable levels and in two cases it became undetectable. However, in two cases, who had earlier undetectable levels, detectable but lower levels were observed. Circulating TNF- α levels in controls and cases before and after treatment are presented in figure 1.

Figure 1. Serum TNF- α levels in cases and controls.

Our study revealed detectable levels of TNF- α in blood in only limited number of patients (6 out of 22) with tuberculosis both before and after treatment. In a similar study by Barlan et. al. 13.6% of children with active pulmonary tuberculosis had measurable serum TNF- α levels (5). There could be several reasons for the non-detection of TNF- α in blood which includes short half life (6), presence of circulating inhibitor (7) and degradation during storage. In the present study, serum samples were stored at -20°C as in previous studies (5), and were analyzed within two months after collection of serum. Some authors have advocated the addition of a protease inhibitor- aprotinin to serum samples in order to inhibit degradation of TNF- α (8), which has not been supported by many others (5). It is possible that local production of TNF- α may participate in an auto-amplification process that results in high local cytokine concentration, while minimizing release of cytokine into the systemic circulation. Very high concentrations have been observed in pleural fluid (9) and broncho-alveolar lavage fluid (10), necessitating estimation of the cytokine at the site of disease as opposed to serum.

Three patients in the present study who had very high serum TNF- α levels were similar to rest of the

patients in terms of clinical and laboratory features. Possibly they may be genetically predisposed to produce increased amounts of the cytokine as has been observed in the association of HLA haplotype to TNF- α production (11).

There was a trend towards decreased TNF-alpha levels after two months of anti-tubercular therapy in six patients in whom they were measurable before therapy along with clinical improvement, possibly because of fall in bacterial load following intensive therapy and subsequent decline in T-lymphocytes and macrophages activation.

The present study has the limitation of small sample size though care was taken to ensure that basal sample was collected prior to initiation of therapy. There was a trend towards younger age, shorter duration of symptoms, presence of fever and anorexia, and high ESR, in patient with high serum TNF- α levels. Further studies with larger sample size are required to evaluate the role of genetic predisposition and strain of *Mycobacterium tuberculosis* in patients with very high circulating TNF- α level, which will provide insight into the role of cytokines in the pathogenesis of tuberculosis.

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