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Research Article

Serum Tumor Necrosis Factor Alpha (TNF-A) Levels after Anti-Tuberculous Therapy

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Abstract: The monocyte/macrophage-derived cytokine, Tumor Necrosis Factor Alpha (TNF-α) plays a central role in effective granulomatous immunity to *Mycobacterium tuberculosis* infections in humans. The present study was conducted to document changes in the serum TNF-α level of patients after varying durations of anti-tuberculous therapy as compared to normal healthy controls. A comparative study was conducted at Ayub Teaching Hospital Abbottabad from January to June 2009 by selecting 64 patients of pulmonary tuberculosis undergoing standard anti-tuberculous therapy of varying durations; seventeen normal healthy individuals acted as controls. Along with other clinical and laboratory indices of disease, the serum TNF-α level was measured from patients by ELISA and compared to controls. The mean serum TNF-α levels of patients were significantly decreased as compared to controls ($p<0.001$); moreover, serum TNF-α levels continued to decline with increasing duration of therapy. In Conclusion Serum TNF-α level of patients undergoing anti-tuberculous therapy should be monitored to prevent significant decreases from normal levels in order to prevent immunosuppression and enhanced risk of opportunistic infections.
Keywords: Tumor Necrosis Factor Alpha (TNF-α), Pulmonary tuberculosis, *Mycobacterium tuberculosis*, anti-TB therapy.

INTRODUCTION

The WHO declared Tuberculosis (TB) as a global emergency in 1993 [1] based on the fact that it is the leading cause of deaths due to infectious diseases worldwide, killing an estimated 3 million people annually. The WHO further estimated that unless controlled, TB death toll would rise one million annually. Tuberculosis kills more people than AIDS, malaria, diarrhoea, leprosy and all other tropical diseases combined. The problem is further compounded by the increased susceptibility to tuberculosis by the presence of HIV/AIDS and the emergence of drug and multi-drug resistance in patients with TB.

As only 10% of people with primary infection go on to develop overt tuberculosis, the immune response to the disease is generally good. However, what appears to be happening is a dysregulation of the immune response, particularly in terms of the type of cell response to the disease. It is well known that Th1 type helper T lymphocytes are primarily involved in the initial cellular response to infection; after excessive antigen challenge, these cells may later on involve Th2 type cytotoxic T lymphocytes by secretion of cytokines [2]. However Th1 & Th2 lymphocytes secrete a potent cytokine, Tumour Necrosis Factor alpha (TNF-α), which has the potential of causing tissue damage if secreted in unregulated amounts. The critical phase is the 'switch over' from Th1 to a Th2 type response – if excessive numbers of Th2 cells are recruited, the result may well be tissue damage in organs undergoing tuberculous infection.[2] In addition TNF-α also renders cells (including macrophages) very sensitive to apoptosis, which further damages the macrophage-mediated arm of the immune system that is normally vital in controlling the spread of *Mycobacterium tuberculosis*. Aggravating immunosuppressive factors such as malnutrition, stress, glucocorticoids and other opportunistic infections tend to recruit more Th2 lymphocytes with resultant increased tissue damage.

Tumour Necrosis Factor (Cachectin) [3] a 17 KD polypeptide cytokine produced mainly by cells of monocyte/macrophage, neutrophilic and lymphocytic lineages, has been implicated in the pathogenesis of local and systemic inflammatory reactions. It is a primary mediator in the pathogenesis of infections, injury and inflammation and also in the beneficial processes of host defence and tissue homeostasis. Depending on its concentration, duration, cell exposure and presence of other mediators in the cellular environment, the net biological effects of this peptide regulatory factor may be ultimately beneficial or injurious to the host. It is well known that acute systemic release of TNF-α causes septic shock and tissue injury. It is also responsible for such systemic effects as fever, weight loss, night sweats, raised ESR, etc. in patients with active tuberculosis. It has a pivotal role to play in the formation of granulomas and caseation necrosis, so characteristic of a successful immune response to M. Tuberculosis infection [4].

In animal models, particularly mice, TNF-α has a potent beneficial role in the immune response to tuberculosis and blocking TNF-α by a variety of means makes these animals more susceptible to acquiring tuberculosis [5]. It is felt that high levels of TNF-α is essential to an effective microbicidal immune response and granuloma formation in tissues [6, 7].

The role of TNF-α has also been studied in various chronic infections such as tuberculosis, leprosy, rheumatoid arthritis and Crohn's disease. It has been implicated as a mediator of tissue injury in advanced stages of these diseases and may be a primary mediator of disease progression as well. Anti TNF-α monoclonal antibodies (Infliximab, Remicade) have been found clinically beneficial in the treatment of Crohn's disease, ankylosing spondylitis and rheumatoid arthritis. However, it is alarming to note that patients receiving Infliximab therapy have an increased frequency of developing latent tuberculosis or reactivating their tuberculosis, as well as other opportunistic infections [8-10].

The present study was undertaken to see the variations in serum TNF-α levels in patients of pulmonary tuberculosis (PTB) as compared to controls and in response to various durations, stages and responses to therapy. Moreover it was intended to seek associations if any, between serum TNF-α levels and other indices like sputum positivity, ESR, leukocytosis and Hb levels.

PATIENTS AND METHODS

This comparative study was conducted at Ayub Teaching Hospital Abbottabad (ATH) from January to June 2009. Patients were taken by convenience sampling from the Outpatients Department (OPD) as well as Pulmonology Ward of the Department of Medicine at ATH and comprised diagnosed cases of pulmonary tuberculosis under routine anti-tuberculous therapy (standard DOTS regimen). Controls were normal people with no evidence of clinical or laboratory disease.

Diagnostic criteria

Tuberculous patients were diagnosed on a combination of clinical and laboratory criteria. These included fever, night sweats, cough, weight loss, chest X-rays, sputum examination for acid fast bacilli (AFB), raised ESR, Hb levels, leucocytosis, differential leucocyte count and absolute lymphocyte count as well as exclusion of other mimicking conditions such as other bacterial, viral or fungal infections. Control subjects were assessed clinically, as well as by relevant investigations to rule out pulmonary or other forms of tuberculosis and other diseases.

Data were collected after informed consent, by specially constructed Performa utilizing direct interview as well as perusal of patients' hospital records. For estimating serum TNF-α and other haematological indices, 5 ml of venous blood was taken, of which 2 ml was allowed to clot, while the remaining was transferred to anticoagulant container for haematology investigations. All serum samples were stored at -20^0C till performance of laboratory tests. Serum Assay was performed using MaxSignal™ Human Serum TNF-α test kit supplied by Bioo Scientific Corporation, Texas, USA with an assay range from 8-512 pg/ml and a sensitivity of 4 pg/ml. Standard 96-well ELISA was performed following the manufacturer's instructions as supplied in the kit manual. Absorbances were read at A450 nm and converted into serum values by using the appropriate regression formula obtained from plotting of standards supplied with the kit.

Data analyses were performed by entering variables into SPSS version 15.0. Routine calculations such as frequencies, ratios, percentages, means and S.D. were performed. Significant differences between groups were tested for by the Chi square test for qualitative variables and the Student's T test for quantitative variables. A p value ≤ 0.05 denoted significance. Regression analysis was performed to document associations between variables of interest.

RESULTS

During the study period, 64 patients of pulmonary tuberculosis were enrolled in the study; these included 60 cases of previously diagnosed disease currently on DOTS therapy from 1 – 8 months and 4 cases of old PTB who had completed their therapy. Seventeen normal persons gave their consent to act as controls.

Data of both controls and patients are provided in tables 1-3 and figures 1&2. Fifty nine (92.2%) of patients belonged to Abbottabad, the remaining were from districts Haripur and Mansehra; all the controls were from Abbottabad. The patient group had 30 (47%) males and 34 (53%) females while the controls had 10 (59%) males and 7 (41%) females. The ages of patients ranged from 6 – 75 years (mean 29.02 ± 17.21 years), whereas those of controls ranged from 24 – 40 years (mean 30.12 ± 4.86 years).

Table 1: Frequency distribution of demographic data of controls and patients

Sl. No.	Variables	Controls (n=17)	Patients (n=64)
1.	Gender		
	Males	10	30
	Females	07	34
2.	Age groups (years)		
	5 – 14	-	11
	15 – 29	09	31
	30 – 44	08	08
	45 – 59	-	09
	60 – 74	-	04
	≥ 75	-	01
3.	Districts		
	Abbottabad	17	59
	Mansehra	-	03
	Haripur	-	02
4.	Duration of therapy (months)		
	1 - 2		45
	3 - 4	N.A.	07
	5 - 6		02
	7 - 8		10

Table 2: Frequency distribution of laboratory values of controls and patients

Sl. No.	Variables	Controls (n=17)	Patients (n=64)	P value
1.	Hb levels (g/dl)			
	8.0 – 9.9	-	16	
	10.0 – 11.9	04	31	0.002
	12.0 – 13.9	11	16	
	14.0 – 15.9	02	-	
	≥ 16.0	-	01	
2.	TLC (/cmm)			
	5500 – 7499	-	17	
	7500 – 9499	04	36	0.01
	9500 – 11499	05	11	
	11500 - 13499	07	-	
	≥ 13500	01	-	
3.	ESR (/1 hour)			
	5 – 24	17	-	
	25 – 44	-	10	< 0.001
	45 – 64	-	28	
	65 – 84	-	16	
4.	L.N. Histology			
	Langhan's Giant Cells		29	
	Granulomas	N.A.	34	N.A.
	Caseous necrosis		17	
	Reactive hyperplasia		30	
5.	Sputum ZN stain			
	AFB Present	N.A.	01	N.A.
	AFB Absent		63	
6.	Serum TNF-α (pg/ml)			
	3.0 –12.9	01	45	
	13.0 – 22.9	12	18	0.097
	23.0 – 32.9	03	01	
	33.0 – 42.9	01	-	

Table 3: Distribution of mean (± S.D.) laboratory values of controls and patients

Sl. No.	Variables	Controls (n=17)	Patients (n=64)	P value
1.	Age (years)	30.12 ± 4.86	29.02 ± 17.21	N.S.
2.	Hb levels (g/dl)	12.60 ± 0.89	10.66 ± 1.6	< 0.001
3.	TLC (/cmm)	10829.41 ± 1578.67	8412.03 ± 1412.99	< 0.001
4.	ESR (/1 hour)	11.82 ± 4.40	57.42 ± 11.69	< 0.001
5.	Serum TNF-α (pg/ml)	19.89 ± 6.42	12.60 ± 4.29	< 0.001

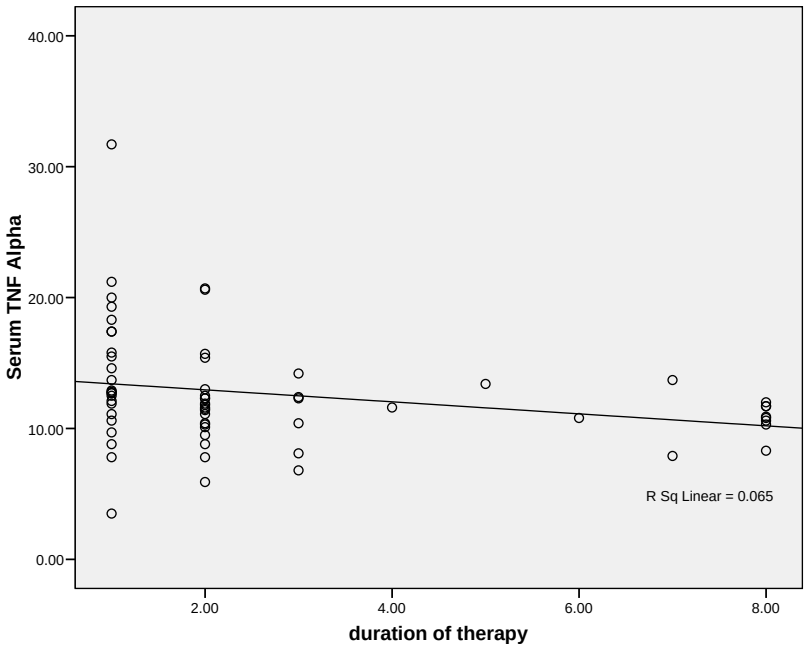

Fig. 1: Scattergram of duration of therapy and serum TNF alpha levels of patients (n=64) showing a negative linear correlation which is significant (r = -0.255, p=0.042)

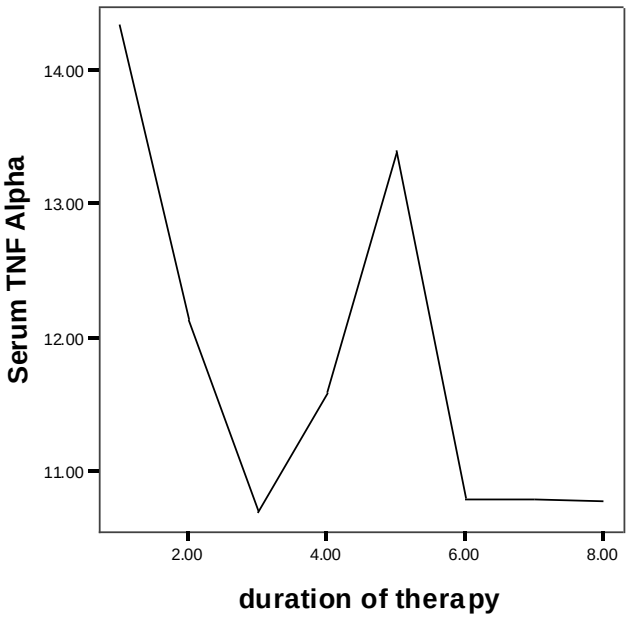

Fig. 2: Plot of serum TNF alpha with duration of therapy (in months) showing the progressive decrease over 8 months of therapy

Of the patients, duration of DOTS therapy ranged from 1 – 8 months, with a mean duration of 2.76 ± 2.38 months. Twenty four patients (37.5%) had therapy for 1 month, 21 patients (32.8%) for 2 months, 6 patients (9.4%) for 3 months, 1 patient (1.56% each) each for 4, 5 and 6 months, 2 patients (3.12%) for 7 months and 8 patients (12.5%) for 8 months.

The haemoglobin levels of patients ranged from 8.0 – 18.0 g/dl, while those of controls ranged from 10.0 – 14.0 g/dl (p=0.002); the mean haemoglobin levels of patients were 10.66 ± 1.6 g/dl while those of controls was 12.60 ± 0.90 (p <0.001).

Total leukocyte counts (TLC) of patients ranged from 5600–11200 leukocytes/cmm while those of controls ranged from 8000–13800 leukocytes/cmm (p=0.01); the mean TLC of patients was 8412.03 ± 1412.99 leukocytes/cmm while those of controls was 10829.41 ± 1578.67 leukocytes/cmm (p <0.001).

The Eyrthrocyte Sedimentation Rate (ESR) ranged from 30-80 mm/1st hour in patients and from 6-23 mm/1st hour in controls (p <0.001); the mean ESR of patients was 57.42 ± 11.69 mm/1st hour while for controls it was 11.82 ± 4.40 mm/1st hour (p <0.001).

Serum TNF-α levels of patients ranged from 3.50-31.70 pg/ml and of controls ranged from 12.60-37.70 pg/ml (p=0.097); the mean serum TNF-α levels of patients was 12.60 ± 4.29 pg/ml and of controls was 19.89 ± 6.42 pg/ml (p <0.001).

Histopathological investigations revealed that diagnostic granulomas were present in 34 (53.12%) patients and were not observed in 30 (46.87%) patients who showed reactive hyperplasia. Moreover, 29 (45.31%) patients had Langhan's Giant Cells in the lymph nodes while the remaining 35 (54.69%) did not show such Giant cells. Caseation necrosis within granulomas was evident in 17/34 (50.0%) patients.

The Ziehl-Nielsen (ZN) stain for acid-fast bacilli (AFB) in sputum was positive in only 1 (1.56%) patient.

Regression analysis revealed a significant negative correlation between duration of DOTS therapy and serum TNF-α levels of patients (Pearson's r=-0.255, p=0.042). Other correlates of the serum TNF-levels of patients (ESR, Hb, TLC, caseous necrosis) showed non-significant correlations or no correlations.

The mean serum TNF-α levels of patients showed a progressive decrease with duration of treatment from 1 month to 8 months, so that after one month the mean level was 14.33 ± 5.3 pg/ml and after 8 months the mean level was 10.78 ± 4.29 pg/ml; however this was not statistically significant (p=0.058). The rate of change was calculated as a decrease of 5.7143 pg/ml per month.

In addition, the other indices of disease such as the ESR (60.88 ± 11.33 mm/1st hour versus 51.88 ± 10.67 mm/1st hour, p=0.058), Hb (10.86 ± 1.89 g/dl versus 10.62 ± 1.06 g/dl, p=0.74) and TLC (8138.33 ± 1453.56 leukocytes/cmm versus 8825.0 ± 1143.62 leukocytes/cmm, p=0.235) also did not show significant changes with duration of therapy when their means at 1 month and at 8 months were compared.

DISCUSSION

The present study was designed the effect of antituberculous therapy on serum TNF-α levels. The finding of decreased serum TNF-α levels of patients as compared to controls is thought-provoking and the subject of further debate. Many studies have documented findings of increased as well as decreased serum TNF-α levels in patients of pulmonary tuberculosis. The exact reason for these disparate findings is not clear, though some authors have attributed decreased levels to either the effects of anti-tuberculous therapy, or to patients suffering from drug-resistant *Mycobacterium tuberculosis*, where the decreased TNF-α levels are postulated to contribute to persistence of bacteria in the tissues. Nevertheless a sequence of events is generally agreed upon, in that the initial Th1 response to tuberculous infection entails active secretion of TNF-α by monocyte/macrophage cells and lymphocytes, which successfully eradicates the organisms and results in an organized granuloma formation in tissues. After this initial phase, containment results in decreases of TNF-α secretion due to curtailment of the Th1 response or its switch-over to Th2 type response with lesser TNF-α secretion. However Th2 cells can also secrete TNF-α and if this response persists, further tissue damage and long range effects like extensive necrosis and scarring may result. The exact modulation of the Th1/Th2 response is still an area of intense debate and factors such as Interferon gamma (IFN-γ) [11], TNF-α, [12] and IL-10 [13] have been implicated. In fact some studies have recommended that levels of IFN-γ, TNF-α and IL-10 be used as indicators of active or progressive disease [14]. Moreover polymorphisms in the genes for TNF-α [12], IL-10 [13] and IFN-γ [11] have been reported in patients of tuberculosis, with the implication that these polymorphisms increase susceptibility to the disease as well as play a role in the clinical severity of disease. The IFN-γ/IL-10 ratio has been advocated as a clinical index of disease severity [15].

Experimental data from mice indicate that TNF-α mediates its cellular apoptotic/necrotic effects through toll like receptors. In mice at least, these receptors have a negative effect on TNF-α mediated effects, and mice with deficient TLRs show rapid death due to massive liver necrosis, accompanied by

increased TNF-α secretions by macrophages/monocytes with raised serum levels of TNF-α [16].

The question of significantly decreased serum TNF-α levels in tuberculosis patients in this study, as compared to controls, raises the possibility that it was the response of anti-TB therapy, as all patients were undergoing active DOTS treatment. Moreover, it was seen that a progressive decrease in serum TNF-α levels occurred in patients as the duration of therapy increased from one month to 8 months. Though there is no mention in the literature of any of the anti-tuberculous drugs causing decreases in serum TNF-α levels, the phenomenon is well known after treatment [17, 18] and is attributed to microbicidal drug activity resulting in decreased *Mycobacterium tuberculosis* antigen in the body.

This explanation would have been reinforced if supported by other evidence of disease amelioration such as the ESR, TLC and Hb levels; however these showed no significant changes over the 8 months of therapy. This raises the possibility that these patients may still have been immunosuppressed due to therapy as well as low TNF-α level, which may have contributed to some low level undetectable infections or other events.

Some authors have reported decreased serum TNF-α levels as a primary event in tuberculous patients as well [19]; moreover there is evidence of decreased secretion of TNF-α from challenged monocytes/macrophages in culture when cells were obtained from such patients. Such findings have been attributed to genetic defects in chemokine secretion or modulation by macrophages and other cells [11, 12, 20].

Decreased serum TNF-α levels in patients undergoing anti-tuberculous treatment raises the possibility of monitoring the treatment by measuring serum TNF-α and other cytokines. This is important because decreased TNF-α levels can have adverse consequences for the patients, raising the risk of re-acquiring or reactivating tuberculosis, as happens in patients of Rheumatoid arthritis, Crohn's disease, Ankylosing spondylitis, Behcet's disease, etc., who are treated with Infliximab. Moreover these patients are at risk of acquiring other diseases such as fungal, nosocomial or opportunistic infections, which are normally kept in check by adequate TNF-α response. At present there are no guidelines as to what sort of cytokine levels should be maintained during anti-TB therapy to offset this disadvantage. Some studies are already looking into this aspect and recommend that some cytokine markers be routinely monitored during anti-TB therapy; however these are early studies and the matter will have to be decided by further large scale sophisticated research work to determine cutoff levels that pose a risk for developing immunosuppression due to decreased serum TNF-α levels.

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