Level of Interferon Gamma in the Blood of Tuberculosis Patients

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ABSTRACT

Background: Interferon gamma (IFN-γ), a cytokine produced by a variety of cells is involved in the immune response against *M. tuberculosis*. It activates the production of other cytokines and molecules that kill mycobacterium. IFN-γ also has diagnostic role in identification of active and latent tuberculosis. Objective: To determine the level of IFN-γ in the blood of TB patients. **Methods:** Ninety-one subjects were selected, including 54 active TB patients and 37 healthy controls. Among 54 TB patients, 27 had confirmed TB and 27 were clinically diagnosed as having TB. IFN-γ concentration was determined in their blood by an ELISA technique. **Results:** In TB patients, Mean $\pm$ SD of IFN-γ was 48.69 $\pm$ 28.78 pg/ml while it was 12.99 $\pm$ 5.70pg/ml in the control group (p <0.001). Significant differences in the level of IFN-γ were observed among confirmed TB patients, clinically diagnosed TB patients and the control group (Mean $\pm$ SD 59.68 $\pm$ 28.78, 36.85 $\pm$ 24.76 and 12.99 $\pm$ 5.70 pg/ml, respectively). Furthermore, a significant negative correlation was observed between the concentration of IFN-γ in TB patients and the duration of anti-tuberculosis therapy. **Conclusion:** IFN-γ level was high in both clinically diagnosed and confirmed TB patients as compared to a control group. Measurement of IFN-γ production is helpful to diagnose active tuberculosis, but further research is required.

Keywords: ATT, ELISA, IFN-γ, MTB, TB

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INTRODUCTION

Tuberculosis (TB) is a fatal infectious disease and a leading cause of mortality and morbidity, worldwide. In healthy individuals, immune system controls the mycobacterium tuberculosis (MTB) infection and the organism remains dormant for some time. In about 10% of individuals, reactivations of latent bacteria proceed to active TB infection (1). Cell mediated immunity plays an important role in preventing the spread of MTB and provides a local immunological environment for the communication and cross talk of immune effecter cells (2). Lysosomal enzymes of macrophages such as acidic hydrolases degrade MTB, but the organism may develop mechanisms to evade the host immune response. One of such mechanisms is the prevention of the fusion of phagosome with the lysosome, resulting in the bacterial survival in the macrophage for a longer period (3). MTB also secretes superoxide dismutase and catalase that are antagonistic to reactive oxygen intermediates (RIOs), thus protecting it from superoxide toxicity (4). Modulation of the antigen presentation by the bacteria is another way to evade cytotoxic T cells (5). Interferon-gamma (IFN-γ) is a soluble protein secreted by a number of immune cells and is a dimeric cytokine that belongs to the interferon family. At the site of MTB infection, it is the major cytokine of Th-1 cells, therefore, as a pro-inflammatory immune response, it protects against MTB (6). IFN-γ activates macrophages to kill intracellular mycobacteria. *In-vitro* cultures of IFN-γ treated macrophages showed a significant reduction in the number of MTB's (7). It has been observed that after disrupting IFN-γ gene, the mice subjected to a sublethal dose of intravenous MTB progressed to disseminate TB (8). IFN-γ knockout mice developed neither mature granulomas nor protective immunity after infection with a virulent MTB strain (9). Diagnostic significance of IFN-γ in both latent and active TB has been reviewed in different studies. Enzyme linked immunosorbent assay (ELISA) for the detection of IFN-γ provides information about how much cytokine is being secreted at a given point and this technique has been found useful in differentiating malignant from tuberculous pleural effusions (10). The present study was carried out to determine the level of INF-γ in the blood of pulmonary TB patients regardless of their status of the disease; i.e. whether active, inactive, acute or chronic, and to compare the levels of INF- γ between TB patients and healthy controls.

SUBJECTS AND METHODS

The study was approved by the Advanced Studies and Research Board of the University of Health Sciences (UHS) and by the Ethical Review Committee of Shalamar Hospital, Lahore. Blood samples of TB patients were collected from Shalamar Hospital and laboratory measurements were performed in the Department of Immunology, UHS, Lahore. The study was an analytical cross sectional study which was performed from December 2007 to October 2008. Ninety one subjects comprising of 54 TB patients and 37 healthy volunteers as a control group, matched by gender, age and socioeconomic status were recruited from the same city. Among TB patients, 25 (46.30 %) were males and 29(53.70%) were females, while in the control group, 25 (67.60 %) were males and 12

were (32.40 %) females. The age range of TB patients and the controls was 15-60 years, and 20-54 years respectively.

Twenty-seven (50%) patients had confirmed TB which included 24 (44.44%) with Zeil Nielsen stain positive sputum and 3 (5.56 %) were biopsy/granuloma positive cases. The remaining 27 (50 %) were clinically diagnosed TB patients. Eleven (20.37 %) TB patients were taking anti-tuberculosis therapy (ATT) for not more than 3 months while 43 (79.62%) were newly diagnosed. Written informed consent was obtained from all the participants before sample collection. Inclusion criteria included either sex between 15-60 years of age, newly diagnosed and known TB patients on treatment for less than three months. For the control group, individuals without history of chest infection in the last two-three weeks were included. Exclusion criteria involved pregnancy, immuno-proliferative or autoimmune disorders, malignancy, allergy, immunosuppressive therapy and TB patients on ATT for more than 3 months. Sample Collection: Five (5) ml of venous blood was drawn and added into 2 sterile vacutainers (BD) containing K2- EDTA, and the tubes were transported to the Department of Immunology, UHS in an ice box without delay. One of the tubes was used for erythrocyte sedimentation rate and complete blood count on Sysmex automated hematology

analyzer (Sysmex 1000X-i) soon after reaching the laboratory, while the other tube was centrifuged and the plasma was separated and stored at -80°C for the detection of IFN-γ by quantitative sandwich enzyme immuno-assay kits (DIACLONE diagnostics, France). Analytical sensitivity of the kit was 0.25pg/ml. The specificity as mentioned by the manufacturer, there was no cross reactivity with other autoantigens, and the detection limits were between 0.25-400pg/ml. The assay was performed according to the manufacturer's directions. Data obtained were analyzed using statistical package for social sciences (SPSS16.0). Mean $\pm$ SD was determined for both quantitative and the qualitative variables and frequencies and percentages were calculated. Independent sample t test was applied to observe group mean differences. Pearson correlation test was applied to observe correlation between quantitative variables and Pearson Chi-square test was used to observe correlation between qualitative variables. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Levels of interferon-gamma in the blood of tuberculosis patients were significantly high (*p*value < 0.001) compared to the control group. The mean $\pm$ SD of IFN-γ (pg/ml) of confirmed TB patients and clinically diagnosed TB patients is given in Table 1.

Table 1: Level of interferon gamma in confirmed TB patients and clinically diagnosed TB patients

Parameter	Confirmed TB patients (n=27) Mean $\pm$ SD	Clinically diagnosed TB patients (n=27) Mean$\pm$ SD	*p* Value

IFN-γ (pg/ml)	59.68 + 3.21	36.85 + 4.92	<0.001

Mean level of IFN-γ in confirmed TB patients was higher than that of the clinically diagnosed TB patients (Fig. 1). Also, the mean IFN-γ level of clinically diagnosed patients was higher than that of the control group and the difference was statistically significant between the two groups (*p* value < 0.001) (Fig. 1).
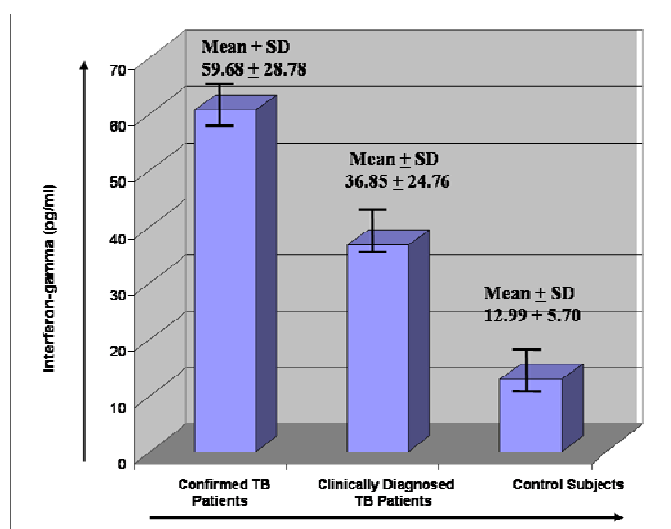

Figure 1. Mean + SD of interferon-gamma of confirmed TB patients (n=27), clinically diagnosed TB patients (n=27) and control group (n=37)

The mean ± SD of ESR, Hemoglobin, total leukocyte, neutrophil, lymphocyte and monocytes count of TB patients and the controls are shown in Table 2.

Table 2: Comparison of laboratory parameters of TB patients and the healthy controls

Parameters	TB Patients Mean + SD	Control Mean + SD	*p* Value
ESR (mm/hour)	62.83 + 26.82	8.89 + 3.15	< 0.001
Hemoglobin (g/dl)	11.40 + 1.67	14.00 + 1.41	< 0.001
Total leukocyte Count (x 10^3/µl)	8.45 + 3.85	7.64 + 1.96	0.248
Neutrophils (%)	67.07 + 9.28	57.52 + 6.53	< 0.001

Lymphocytes (%)	24.20 $\pm$ 8.64	31.54 $\pm$ 6.32	< 0.001
Monocytes (%)	8.38 $\pm$ 4.26	8.55 $\pm$ 1.98	0.818

In TB patients, a statistically significant negative correlation (p <0.001 and r = -0.544) was observed between the level of interferon-gamma (pg/ml) and the duration of antituberculosis therapy. It should be noted that with an increase in the duration of antituberculosis treatment (ATT), the serum interferon-gamma level decreases (Fig. 2).
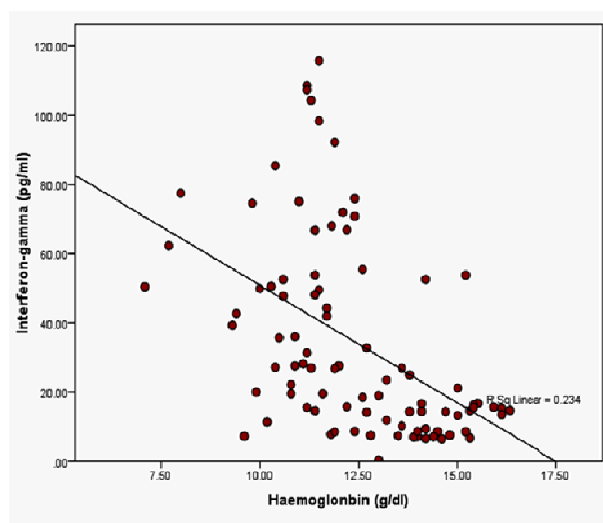

Figure 2. Correlation of interferon-gamma with the duration of anti-tuberculosis therapy

A statistically significant negative correlation (p <0.001 and r = -0.433) was observed between the level of serum interferon-gamma and the hemoglobin concentration. (Fig. 3)

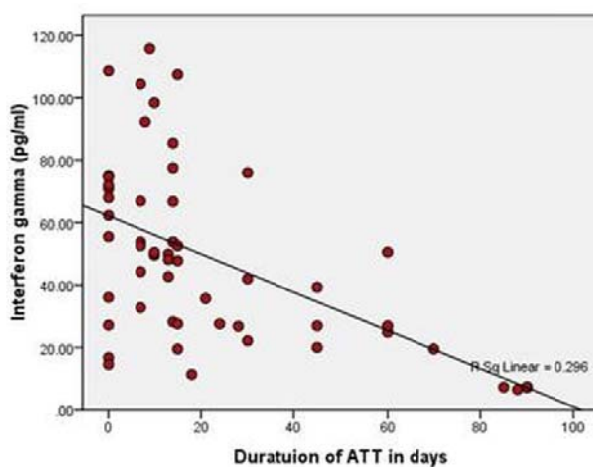**Figure 3.**   Correlation of interferon-gamma with hemoglobin concentration

DISCUSSION

This study demonstrated a significant difference (*p* value < 0.001) in the levels of IFN-γ in the blood of tuberculosis patients (n= 54) as compared to that of the control subjects (n=37). Mean IFN-γ level of confirmed TB patients was higher than that of the clinically diagnosed TB patients. This may be due to the fact that most of clinically diagnosed TB patients were on anti tuberculosis therapy and therefore the healing effect on granuloma could reduce the number of local and circulating IFN-γ producing activated T cells. This explanation was supported by the observation of a negative correlation between the levels of interferon gamma and duration of ATT in TB patients which demonstrated that drug therapy caused a decrease in the level of IFN-γ. At the same time, it was difficult to understand the negative correlation observed between the level of INF-γ and the blood hemoglobin of the patients. A similar therapeutic effect was observed by *Aiken et al* and *Pai et al* who demonstrated a similar response against ESAT-6 and CFP-10 antigens of MTB (11, 12).There are studies in which investigators have reported increased levels of the IFN-γ in pleural effusion (13) or in the sputum (14) and observed a correlation between IFN-γ and disease activity. Some of the earlier studies have focused on the comparison of the systemic IFN-γ level and the results of tuberculin skin testing (TST) (15), but we did not perform this comparison because the majority of our community is randomly immunized with BCG vaccination which could produce variable and unpredictable results. Moreover skin test remains positive for years in healthy individuals after BCG vaccination (16).

The hemoglobin concentration of TB patients showed a statistically significant association with the level of IFN-γ. The percentage of lymphocyte in TB patients was relatively low as compared to the control group. Since most of our patients admitted to the hospital were of low socio economic status, immune-compromised status could be the reason for low lymphocyte count. However, it is probable that the lymphocytes of these patients were functionally active as indicated from the levels of IFN-γ in their blood. Although number of monocytes is important in the pathogenesis of TB, but random BCG immunization in the community might have lead to almost similar number of monocytes in the active TB and the control groups. In some of the earlier work, specific mycobacterial antigens like ESAT6 and CFP-10 were used to stimulate IFN-γ production (17). Although we did not use such specific antigens in our study, but significantly high levels of IFN-γ were found in the blood of our TB patients as compared to the control group. Clinically diagnosed TB patients had a lower level of IFN-γ as compared to AFB/granuloma positive confirmed TB patients. Probably different stages of MTB infection in the clinically diagnosed patients could be the reason for these findings. Further, it is difficult to report the sensitivity and specificity of IFN-γ-ELISA procedure because a gold standard diagnostic technique such as culture of MTB was not included in the study.

Currently, the level of IFN-γ is being used to diagnose active TB infection, therefore where the prevalence of TB is low and clinical findings suggest TB, then a positive test may indicate active TB infection. However, in TB endemic countries like Pakistan, positive test results would be less meaningful and could indicate only a latent infection. Both *in- vitro* and *in-vivo* studies are required to establish the immuno-regulatory and the diagnostic role of this cytokine in our population. IFN-γ assay in blood and other body fluids could potentially provide means of diagnosis of suspected TB patients.

ACKNOWLEDGMENTS

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