

## Cytokines as Biomarkers in the Diagnosis of MDR TB Cases

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### Abstract

**Background:** Cytokines play important roles in many physiological responses and are involved in the pathophysiology of a wide range of diseases. Because of their regulatory nature, their potential as therapeutic agents has been explored. Cytokines have pleiotropic and regulatory effects and participate in the host's defense and in inflammatory and tissue reparation processes.

**Material and Methods:** The study was conducted at the Department Of Microbiology, J.N.M.C, Aligarh, India. Different cytokines levels were measured in 65 samples of TB patients of whom 30 (46.1%) were new TB cases and 35 (53.8%) were suspected multi drug resistant (MDR) TB cases along with 15 BCG vaccinated healthy controls by ELISA (Diacclone France).

**Results:** The serum concentration of TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL10 & IL-12p70 in patients with active TB & MDR TB was elevated than in treatment patients (P < 0.001) and in controls (P < 0.001).

**Conclusions:** These cytokines can be used as a biomarker for the diagnosis of MDR TB cases and for the prediction of differential treatment responses in new and under-treatment TB cases.

**Keywords:** Cytokine; MDR TB; Mycobacterium

### Introduction

A variety of chemokines and cytokines are secreted from infected cells and tissue macrophages. TNF- $\alpha$  increase early in the disease and take part in the pathogenesis and prevention of mycobacterial infection. TNF- $\alpha$  also appears crucial for the formation of Mtb constraining granulomas, infection control and elimination of mycobacteria [1].

Immunologic resistance and susceptibility to intracellular pathogens are mediated by CD4+ T cells with specific patterns of cytokine secretion. Th1 cells that produce gamma interferon confer resistance to infection with Mycobacteria [2].

IL-1 $\beta$  is produced at the site of Mtb infection where IL-1 $\beta$  stimulates a protective pro-inflammatory response. The intense pro-inflammatory response is associated with tissue damage that is neutralized by the release of anti-inflammatory cytokines [3].

Production of anti-inflammatory cytokine IL-10 in response to M tuberculosis may down-regulate the immune response and limit tissue injury, but excessive production of these cytokines may result in failure to control the infection [4].

IL-12p<sub>70</sub> is known to play an important role in anti-TB cell mediated immunity [5] and is the major cytokine for directing primary Th1 differentiation in CD4+ T cells *in vitro* and *in vivo* [6]. The discovery of biomarkers for TB treatment response is therefore important for both clinical practice and for clinical trials of new anti-TB drugs.

T-cell response to an MDR-TB infection in human remains unclear [7]. Most studies on cytokines during TB are from 'in vitro'-stimulated lymphoid cells with few reports on *in vivo* plasma levels [7,8,9]. There is a paucity of data regarding cytokine interplay in MDR-TB patients. With this background, we undertook this study to determine variations in above mentioned cytokines levels in new TB and MDR TB cases.

## Materials and Methods

The present study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University and Aligarh, India. Samples were collected from department of TB & Chest, Jawaharlal Nehru Medical College, AMU and Aligarh. A complete clinical and radiological data was collected. Informed consent was taken from all subjects. The study was approved by the Institutional Bioethical Committee.

1. **Microscopy and Sputum Culture:** Morning sputum samples were collected from the TB patients on three consecutive days. TB diagnosis was based on clinical sign and symptoms and radiological confirmation of pulmonary and extra-pulmonary TB, presence of acid-fast bacilli (AFB) in the sputum smear following Ziehl - Neelsen test. Sputum culture positive for Mycobacterium tuberculosis was confirmed by inoculation of samples on Lowenstein Jensen (LJ) media. The reference strain H37Rv was used as control.
2. **Blood Collection:** 76 sera samples were obtained from patients with active TB before treatment. Patients of all age groups with both pulmonary & extra-pulmonary TB were included. Extra-pulmonary sites included were pleural, lymph nodes, soft tissues, meninges, gastrointestinal, bone and joints and disseminated disease. Sera were also obtained from patients with TB who had been treated for at least 2 weeks, but had not yet completed therapy at the time of blood sampling and from patients who had completed anti-tuberculous therapy. Records of all patients with active TB was reviewed for clinical data such as fever (rectal temperature > 38°C), anorexia, skin test, Chest X-Ray, bacille Calmette-Guérin (BCG) vaccination, direct microscopy, and culture results. Furthermore, 15 sera samples were obtained from persons who had been in close contact with patients with smear-positive TB and from healthy (all skin test-negative) controls.
3. **Evaluation of Chest Radiograph:** All TB patients had undergone plain postero-anterior and lateral chest radiography. Chest radiographs were evaluated for the presence and distribution of signs relating to the active pulmonary tuberculosis that include miliary patterns, cavity, fibro-cavity, segmental consolidation, lobar consolidation, infiltrate and patchy opacity.
4. **Cytokines Assay:** Measurements of all cytokines were performed by sandwich ELISA method and using commercially available ELISA kits. ELISA test was performed following the supplier's instructions. The absorbance values were analyzed by ELISA reader at 450 nm (Thermo Electron Corporation, Vantaa, Finland).
5. **Statistical Analysis:** All statistical analyses were performed using SPSS Statistics (version-20). Receiver operating characteristic (ROC) curve and other performance measures were performed using the statistical software Med Calc (version 10.2.0.0). Pair wise analysis using Chi square test was done to compare differences in cytokines levels between groups of patients. Analysis and results were considered significantly different at ( $P < 0.05$ ).

## Results

In this study 76 patients were enrolled in which 33 (43.42%) were new, 24 (31.57%) were UT, 19 (25%) were MDR TB cases, 1 (1.31%) had pneumothorax, 2 (2.63%) had pyopneumothorax while 1 (1.31%) had diabetes mellitus. None of the patients was HIV positive. 15 BCG vaccinated healthy individuals were included as controls.

TNF- $\alpha$  levels were elevated in new and multi-drug resistant TB patients compared to healthy controls ( $P < 0.001$ ,  $P < 0.001$  respectively) but levels of TNF- $\alpha$  are less significant in under-treatment cases ( $P > 0.001$ ).

The levels of IFN- $\gamma$  & IL-1 $\beta$  were elevated in new and MDR TB cases compared to controls ( $P < 0.001$ ). There was less significant difference between under-treatment patients and controls.

IL-6 & IL-10 levels were significantly elevated in patients with TB during and after treatment compared to controls ( $P < 0.001$ ).

IL-12p<sub>70</sub> and concentration were also increasing in new TB cases and MDR TB cases Compared to Under-treatment and healthy controls ( $P < 0.001$ ).

All these cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-10 & IL-12p<sub>70</sub>) showed no significant variations according to the site of involvement in pulmonary vs. extra- pulmonary TB cases ( $P > 0.001$ ).

### Discussion

Upon stimulation by a pathogen, macrophages engulf the offending particle, and upon its destruction, they present smaller peptide antigens on their surface. These antigens are then recognized by Th1 cells, which in turn secret various cytokines including IF- $\gamma$  IL-12 and TNF- $\alpha$ . These cytokines in turn activate resting macrophages, which trigger the immune response. TNF- $\alpha$  is believed to play multiple roles in the immune and pathological responses in TB [10].

In our study, we found the raised levels of TNF- $\alpha$  in new TB cases compared to healthy controls. Some previous studies have been shown the raised levels of TNF- $\alpha$  in new TB patients compared to healthy controls [11, 12]. Also we found that serum TNF- $\alpha$  levels declined significantly in UT cases ( $P < 0.05$ ). Similarly, Tang, *et al.* [12], Portales- Perez, *et al.* [13] and Kawagnahi, *et al.* [14] found decreased TNF- $\alpha$  levels in TB patients after therapy. Also we found the raised level of TNF- $\alpha$  in MDR TB patients compared to healthy controls. Eumsy, *et al.* [15] also reported association of TNF- $\alpha$  levels with MDR TB.

TH1 cells and natural killer (NK) cells secrete IFN- $\gamma$ , which activates alveolar macrophages to produce a variety of substances involved in growth inhibition and killing of mycobacteria [16]. IFN- $\gamma$  might also improve or augment antigenpresentation, leading to recruitment of CD4+ T-lymphocytes and/or cytotoxic T-lymphocytes, which might participate in mycobacteriakilling [17]. Levels of IFN- $\gamma$  were significantly higher in new and MDR TB patients compared to healthy controls. Decreased levels of IFN- $\gamma$  in under-treatment TB cases have also been reported. Our observations are in agreement with other studies showing the increased levels of IFN- $\gamma$  in new and MDR TB patients. This may be due to the fact that most of clinically diagnosed TB patients were on ATT and therefore the healing effect on granuloma could reduce the number of local and circulating IFN- $\gamma$  producing activated T-cells [18].

IL-1 $\beta$  is produced at the site of Mtb infection where IL-1 $\beta$  stimulates a protective pro-inflammatory response [19]. Increased levels of IL-1 $\beta$  found in our study in new and MDR TB patients. Similarly, Imran, *et al.* found the same observations of IL-1  $\beta$  [20].

An excessive activity of IL-6 inhibits IFN- $\gamma$ , a vital signaling molecule required to activate adaptive immune response and stimulation of the macrophages to ultimately kill or confine Mtb, which is critical to immune protection against Mtb [21]. In our study we showed that IL-6 serum levels were elevated in the vast majority of patients with new TB and MDR TB.

IL-10 in response to M tuberculosis may down-regulate the immune response and limit tissue injury, but excessive production of these cytokines may result in failure to control the infection [22]. In Mtb infection, one of the earliest events of activation of cell-mediated immunity involves the production of IL-12p<sub>70</sub> [23]. We found increased level of IL-10 and IL-12p<sub>70</sub> in new and MDR TB cases compared to controls.

### Conclusion

The present study show that cytokines can be use as biomarkers for the detection of new and MDR TB cases. The above findings are encouraging as they support the concept of host biomarkers for the prediction differential TB treatment responses. We suggest that these cytokines marker at the end of therapy could provide an early indication for discontinuation of therapy, a significant problem for MDR-TB where treatment courses often exceed 2 years.

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