Original Article

# TNF-alpha Levels in Serum and Mononuclear Cell Lysate of Nigerian Tuberculosis Patients at Diagnosis

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## ABSTRACT

***Background and Objectives*:** The need for additional markers for differential diagnosis of drug resistant *Mycobacterium tuberculosis* (*M.tb*) and drug sensitive *M.tb* have been raised by previous studies. Tumor necrosis factor-alpha (TNF-α) produced by monocyte/macrophage, neutrophilic and lymphocytic linkage plays a central role in effective immunity to many disease conditions including *M.tb*. Since *M.tb* either preferential inhabits or transits cells producing TNF-α, it is therefore diagnostically useful to measure the levels of TNF-α in the both mononuclear cell lysate and serum of drug sensitive TB (DS-TB) patients and multi-drug resistant TB (MDR-TB) patients compared with healthy controls.

***Materials and Methods*:** The study was conducted in the Department of Chemical Pathology and Immunology, University of Ibadan, Nigeria; Medical Out-Patients' Department and Multi-Drug Resistant Tuberculosis Center, University College Hospital, Ibadan, Nigeria. TB patients were selected by Consultant Chest physician after detailed laboratory and clinical procedures. The levels of mononuclear cell lysate- and serum- TNF-α were measured using enzyme linked immunosorbent assay (ELISA).

***Results*:** Serum and lysate TNF-α levels were significantly elevated in DS-TB patients ($p < 0.05$) and MDR-TB patients ($p < 0.05$) compared with the control. But serum and lysate TNF-α levels were not significantly different in DS-TB patients compared with MDR-TB patients ($p > 0.05$).

***Conclusions*:** Serum level of TNF-α or lysate TNF-α does not differentiate DS-TB patients from MDR-TB patients and the use of antagonist of TNF-α be handled with caution in TB patients.

## INTRODUCTION

The rise in new cases of *Mycobacterium tuberculosis* (*M.tb*) infection and high annual morbidity and mortality worldwide due to tuberculosis (TB) were associated with difficulty in the eradication of TB, incomplete knowledge of immune responses to *M. tb* and lack of early differential diagnostic marker[1].The study on protective immunity against TB is extensive[34] and it was reported to involve interaction of antigen-specific T cells and macrophages mediated by cytokines produced by these cells[2].During *M.tb* infection, activated macrophages produce TNF-α to recruit more macrophages, other immune cells and stromal cells to aggregate around the infected cells forming granulomas[3]. Within the granuloma, the cell-cell interactions develop to amplify the immune

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responses and enhance TNF-α, MMP-1 and MMP-9 expression in immune cells and stromal cells[4].

TNF-α secreted by activated macrophages has antitubercular action and reduces disease pathology[5]. TNF increases the phagocytic capacity of macrophages and enhances intracellular killing of mycobacterium via the generation of reactive intermediates molecules, effectively synergising with interferon (IFN)-γ[6]. TNF-α is also involved in maintaining formation of granuloma which prevents mycobacterium from disseminating into the blood[7]. These TNF-mediated immune mechanisms may explain the reason for the increased risk of TB in patients receiving anti-TNF agents' treatment.

Previous studies determined cytokine profiles in the sera of TB patients[8]and supernatants of antigen non-stimulated or stimulated peripheral blood mononuclear cells[9, 10]. Moreso, sputum cytokine profile was reported by Riberio-Rodrigueet al[11] as an early clearance of *M.tb*. Since *M.tb* either preferential inhabits or transits cells producing TNF-α, it is therefore diagnostically useful to measure the levels of TNF-α in lysate from these cells which may best reflect the interaction between the host and *M.tb* during active disease. However, knowledge of the TNF-α profile in both sera and monoclear cell lysate of Nigerian DS-TB and MRD-TB patients is lacking. To provide information on immune responses at the local and systemic levels of Nigerians infected with *M.tb*, this study determined TNF-α levels in the mononuclear cell lysates and sera of both DS-TB and MDR-TB patients compared with TB-free apparently healthy controls. The aim is to find out differential diagnostic value of TNF-α in *M.tb* infection and to support or otherwise the proposition thatpatients treated with TNF-α antagonists are at high risk of TB.

## MATERIALS AND METHOD

Patient's recruitment and collection of sera were as recently described elsewhere[12]. Briefly, a total of 90 participants were enrolled for this study. This comprised of thirty (30) multi-drug resistant TB (MDR-TB) patients, thirty (30) drug-sensitive TB (DS-TB) patients and thirty (30) non-TB apparently healthy controls. MDR-TB patients had been previously diagnosed as being infected with isoniazid and rifampicin resistant strains of *Mycobacterium tuberculosis* (Mtb) using clinical history, Chest X-ray and GENE Xpert. These patients were admitted into the MDR-TB centre, University College Hospital (UCH) Ibadan, Nigeria for anti-TB treatment. DS-TB patients were recruited from the Medicine Out-patient Clinic, University College Hospital, Ibadan, Nigeria by a Consultant Chest Physician after confirmation with Microbiological test (sputum smear microscopy), chest X-ray and clinical history.

Five milliliters (5 ml) of blood was drawn from the antecubital vein of each participant. This was dispensed as 2ml into sterile plain sample tubes without anticoagulant to obtain serum after centrifugation at 1000g for 15mins and serum was obtained. Serum was stored at -20°C until analysis. The remaining 3ml was dispensed into lithium heparin tube and mixed with 3ml of Phosphate Buffered Saline (PBS). Lymphoprep(6ml) carefully layered on it and was at 600g for 15mins to obtain mononuclear cells above the mixture of Polymorphonuclear cells and red blood cells. Mononuclear cells obtained were washed, resuspended in Ringers solution, counted and adjusted to 0.5 x $10^6$ cells/ml. Mononuclear cell lysate was obtained by freeze thaw method as described by[15]. Cell suspension was frozen for 15mins at -20°C and thawed at 4°C for 30mins. This procedure of freezing (-20°C, 15mins) and thawing (4°C, 30mins) was repeated to make three cycles.

Microscopic examination confirmed complete disruption of mononuclear cells. Lysate was stored at -20°C until analysis. Enzyme Linked Immunosorbent Assay (ELISA) method was used for the determination of serum and mononuclear cell lysate concentrations of TNF-α as specified by kit manufacturer (Invitrogen Inc., USA).

## RESULTS

Serum and lysate TNF-α levels were significantly elevated in DS-TB patients ($p < 0.05$) and MDR-TB patients ($p < 0.05$) compared with the control. But serum and lysate TNF-α levels were not significantly different in DS-TB patients compared with MDR-TB patients ($p > 0.05$).

Table 1: Comparison of Mean TNF- α Levels (pg/mL) in Serum- and Mononuclear Cell Lysateof MDR-TB, DS-TB and Apparently Healthy Control

| Variables | MDR-TB | DS-TB | Control | p' | p" | p"' |
|---|---|---|---|---|---|---|
| Serum TNF-a (pg/mL) | 30.10±16.36 | 20.86±12.20 | 8.26±2.63 | 0.000* | 0.001* | 0.090 |
| Lysate TNF-α (pg/mL) | 9.08±3.67 | 11.40±4.39 | 5.51±2.43 | 0.006* | 0.000* | 0.128 |

p' MDR-TB compared with control
p" DS-TB compared with control
p"' MDR-TB compared with DS-TB
*Significant at p<0.05

## DISCUSSION

To our knowledge this is the first time that the levels of TNF-α will be described in both mononuclear cell lysate and serum of DS-TB patients and MDR-TB patients. The present data show that serum and lysateTNF-α were raised in both DS-TB and MDR-TB patients compared with non-TB healthy control. This is in constistent with other studies reporting lower levels of TNF-α with treatment of TB[13,14]. TNF-α has been implicated in the pathogenesis of local and systemic inflammatory conditions[16]. Therefore, the increases in TNF-α levels in the sera of TB patients apart from the local site (lysate of mononuclear cells) of production might be a pointer to the systemic inflammation in the TB patients. Thus, reportedly raised levels of other acute phase reactants such as fibrinogen, C-RP, alpha 2-macroglobulin and reduced albumin concentration in Nigerian TB patients supported inflammatory responses in TB patients as indicated by significantly increased levels of serum TNF-α in TB patients considered for this study[17,18].

Apart from being, a primary mediator of injury and inflammation, TNF-α also beneficial effects in host defences, inhibit tumorigenesis and viral replication[21, 22]. But depending on concentration, TNF-α may be injurious if secreted in unregulated amounts[22]. In addition, TNF-α also render cells (including macrophages) susceptible to apoptotic damage[23], further suppressing the macrophage-mediated immune responses to *M. tb* infection. Therefore significantly raised levels of TNF-α in TB patients might be hypothesised to be one of the causes of systemic effects as fever, weight-loss, night sweats, immunosuppression in patients with active TB[24, 25].As previously reported, dysregulation of TNF production has been implicated in Alzheimer's disease, cancer, major depression and psoriasis [26, 27, 28, 29]. This dysregualted TNF-a production might be the case in TB patients considered for this study.

TNF-α antagonists (etanercept, adalimumab, infliximab, golimumab and certolizumabpegol) have been found promising candidates for future clinical applications in many relevant diseases[30]. However, an increased risk of TB has been observed among patients receiving anti-TNF treatments[31].Nevertheless, the association between TNF-α antagonists and an increased risk of TB remains uncertain. However,noanti-tuberculousdrugs were reported to cause decreased serum TNF-α levels[32,33]. It might be conjectured from this study that though excessive TNF-α might be associated with adverse symptoms in TB patients but possibility of using anti-TNF-α be handled wth caution so as not to reduce TNF-α below the level that is protective to *M.tb* infection.

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