Levels of Interleukin-6 In Mononuclear Cell Lysate of Nigerian Pulmonary Tuberculosis Patients

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Abstract

This study reported the changes in lysate interleukin-6 (IL-6) from mononuclear cells in multi-drug resistant pulmonary tuberculosis (MDR-TB) patients and in drug-sensitive pulmonary tuberculosis (DS-TB) patients at diagnosis and during treatment compared with un-infected control. This is to find out if lysate IL-6 has any predictive value for inflammation at diagnosis or during treatment of tuberculosis. Mononuclear cell lysate of blood drawn on the day of diagnosis and on months 2, 4 and 6 of anti-tuberculosis treatment were used for the measurement of IL-6 by ELISA. IL-6 levels of mononuclear cell lysate were significantly reduced in DS-TB patients ($p < 0.05$) and MDR-TB patients at diagnosis ($p < 0.05$) compared with the control. IL-6 levels of mononuclear cell lysate were significantly raised in MDR-TB patients at 2 months and 4 months of anti-tuberculosis chemotherapy compared with the level at diagnosis. Also, IL-6 levels were not significantly different in MDR-TB patients compared with DS-TB patients during treatment ($p < 0.05$). Our results, for the first time, demonstrated that levels of lysate IL-6 from mononuclear cell lysate were increased during treatment of MDR-TB patients but not DS-TB patients.

Keywords: Tuberculosis; Cytokines; Biomarkers; Diagnosis; Chemotherapy.

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Introduction

Mycobacterium tuberculosis (*M.tb*) infection remains a global public health problem in developing countries where there is increase in tuberculosis (TB) prevalence, high rates of new cases associated with mortality annually[1]. The persistence of TB in developing world is due to ability of *M.tb* to succumb host immune responses, poor management of TB programmes, lack of treatment adherence due to long duration of drug use, co-infections and presence of drug-resistant *M. Tuberculosis* strains[2-6]. Additionally, lack of biomarkers to differentiate DS-TB from MRD-TB at diagnosis or absence of biomarkers to predict effective treatment at early stage of treatment are hypothesised by the authors of present study as further reason for persistence of *M.tb* infection.

Till date, monitoring TB treatment relies mainly on sputum culture or smear microscopy status which have their inherent constraints[2,,7] especially in TB patients with HIV co-infection[6] or TB patients with extra-pulmonary disease[8]. Moreover, sputum culture conversion at 2 months was reported not to always predict cure in TB patients[4] and it is a long duration to ascertain treatment response when drug resistance strain of *M.tb* would have manifest or establish itself[5]. These limitations necessitated the need for additional surrogate biomarkers of response to TB treatment.

The present study proposed that cytokine(s) secreted by mononuclear cells will be a suitable biomarker for TB due to intimate association of *M.tb* infection with these host cells. During TB, the interaction *M.tb* with macrophages lead to differential induction and elaboration of several pro-inflammatory including IL-6 and anti-inflammatory cytokines such as IL-10[9].The interplay of these cytokines with immune cells orchestrates progressive effective innate anti-mycobacterial immune response[10]. IL-6 together with TNF-α and IL-1β initiates early pro-inflammatory responses[11], stimulates production of acute-phase proteins (APPs)[12], promotion of T-

cell and B-cell responses[11] and plays a role in the priming of a TB subunit vaccine[13]. IL-6 is produced by a variety of cell types, but the most important sources are monocytes at inflammatory sites and macrophages infected by *M.tb*[8-11,14]; and it has been implicated in the immune-pathogenesis of tuberculosis [11–15]. Saunders et al[16] demonstrated that blood IL-6 is required for the rapid expression of an initial protective IFN-gamma response during *M. tb* infection. IL-6 induces apoptosis, fever and oxidative stress[17,18]. These cells and symptoms are associated with *M.tb* infection[19,20].

The present study investigated the changes in IL-6 from mononuclear cell lysate of DS-TB and MDR-TB patients before treatment, during treatment and at the end of treatment as a means of identifying candidate host markers to predict early treatment outcome or presence of *M.tb* infection. Other study had associated serum levels of IL-6[21-23] and sputum level[24] of IL-6 with effectiveness of anti-TB chemotherapy and *M.tb* clearance. Our results, for the first time, demonstrated that IL-6 from mononuclear cell lysate can be used as a biomarker of mycobacterial infection, either at onset or during treatment.

2. Materials and Methods

This study was approved by University of Ibadan/University College Hospital, Ibadan Joint Ethical Review Committee (No.UI/EC/13/0340).

Patient's recruitment from Medical Out Patients Department and MDR-TB Treatment Center, University College Hospital, Ibadan, Nigeria. 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 millilitres (5 ml) of blood was drawn from the antecubital vein of each participant. Three (3ml) was dispensed into lithium heparin tube and mixed with 3ml of Phosphate Buffered Saline (PBS). The blood samples were taken at diagnosis, 2 months, 4 months and 6 months of anti-tubercular chemotherapy. 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[19]. Cell suspension was frozen for 15mins at -20^{o}C and thawed at 4^{o}C for 30mins. This procedure of freezing (-20^{o}C, 15mins) and thawing (4^{o}C, 30mins) was repeated to make three cycles. Microscopic examination confirmed complete disruption of mononuclear cells. Lysate was stored at -20^{o}C until analysis. Enzyme Linked Immunosorbent Assay (ELISA) method was used for the determination of mononuclear cell lysate concentrations of IL-6 as specified by kit manufacturer (Invitrogen Inc., USA). Student t-test was used to compare two mean values. p-value less than 0.05 was considered significant.

3. Results

3.1. At Diagnosis

IL-6 levels of mononuclear cell lysate were significantly reduced in DS-TB patients ($p < 0.05$) and MDR-TB patients at diagnosis ($p < 0.05$) compared with the control. IL-6 level of mononuclear cell lysate was not significantly reduced in MDR-TB patients compared with DS-TB patients at diagnosis ($p > 0.05$).

3.2. During anti-tubercular Chemotherapy

IL-6 levels of mononuclear cell lysate were significantly raised in MDR-TB patients at 2 months and 4 months of treatment compared with the level at diagnosis ($p < 0.05$). In contrast, IL-6 levels of mononuclear cell lysate were not significantly different in DS-TB patients at 2 months, 4 months and 6 months of treatment compared with the level at diagnosis($p > 0.05$).

4. Discussion

Identification of specific biomarker for TB will pave way for the development of new and

Table 1: Comparison of Mean IL-6 Levels (pg/mL) in Mononuclear Cell Lysateof MDR-TB, DS-TB and Apparently Healthy Control

Time	MDR-TB (n=30)	DS-TB (n=30)	Control (n=30)	p'	p''	p'''
Diagnosis	8.51±3.48	15.06±11.84	29.80±16.56	0.001*	0.034*	0.110
2 months	28.74±15.74	7.48±1.33		[a]0.003*	[a]0.140	
4 months	15.75±8.40	16.16±8.75		[b]0.009*	[b]0.422	
6 months	9.06±6.90	8.34±2.27		[c]0.834	[c]0.266	

*Significant at $p<0.05$

p' MDR-TB compared with control

p'' DS-TB compared with control

p''' MDR-TB compared with DSTB

[a] 2 months post treatment compared with diagnosis

[b] 4 months post treatment compared with diagnosis

[c] 6 months post treatment compared with diagnosis

effective drugs, vaccines and diagnostic or prognostic tests for TB. Therefore, there is an urgent need to have a suitable biomarker. The concept of cytokines to function as biomarkers is well established and has been reviewed[25]. Also, C-reactive protein, an APP, and other markers of inflammation have also been suggested to function as biomarkers of TB[26,27].

In the present study we observed that MDR-*M.tb* and DS-*M.tb* significantly reduced lysate IL-6 level at diagnosis but anti-tubercular chemotherapy enhanced levels of lysate IL-6 in MDR-TB patients only. During tuberculosis, macrophages are the first host cells to interact with *M.tb*. The interaction leads to differential induction and elaboration of several pro-inflammatory including IL-6 and anti-inflammatory cytokines including IL-10. The intricate interplay of these cytokines is thought to orchestrate the induction and progression of an effective innate anti-mycobacterial immune response. IL-6 is produced by a variety of cell types, but the most important sources are monocytes at inflammatory sites and macrophages infected by *M.tb*[13-16] and it has been implicated in the immune-pathogenesis of tuberculosis[20-23]. Saunders et al.[13] demonstrated that IL-6 is required for the rapid expression of an initial protective IFN-gamma response during *M.tb* infection. However, the precise mechanism by which IL-6 mediates protection has not been completely clarified[11,13] and no study has assessed mononuclear cell lysate IL-6 in TB patients.

IL-6 is one of important inductors of the acute-phase response. It is termed endogenous pyrogens because it causes fever and is derived from an endogenous source rather than from bacterial components[9, 10]. Thus, the need to assess the status of IL-6 in the focus of IL-6 production or in the habitat of *M.tb* but not in the systemic circulation.It has been reported that IL-6 plays an important role in protection against murine *M.tb*infection[28,29] due to the influence of the $CD4^+$ T cells response[30]. *M.tb*-infected IL-6-deficient animals show an impaired Th1 response and increased bacterial loads, indicating a requirement for IL-6 in host resistance to *M.tb* infection[28]. IL-6 secreted by *Mtb*-infected macrophages suppresses the responses of uninfected macrophages to IFN-γ[31]. Taking together, IL-6 plays significant roles in host control of *M.tb* infection. Thus, accounting for reduced levels of lysate IL-6 in both groups of our tuberculosis patients at diagnosis compared with control.

In the present study, we found significantly increased levels of IL-6 as treatment progresses in MDR-TB patients but not in DS-TB patients. Reason for the differential elaboration of cytokines by pathogenic and non-pathogenic mycobacteria was reported to be because of differences in the structure of lipoarabinomannan present in the *M.tb* cell wall[32,33]. The differences in the lipoarabinomannan structure have also been associated with the ability of mycobacteria to survive and replicate within macrophages, and to cause a productive infection[32]. The significant increases of IL-6 as drug treatment progresses in MDR-TB patients might be due to the persistence of *M.tb*causing MDR-TB which was likely different from the strain causing DS-TB. Also, since the drugs used by MDR-TB patients were different from the drugs taken by DS-TB patients, it mightbe postulated that the drugs taken by

MDR-TB patients stimulated IL-6 production. Moreso, since IL-6 is an indicator of inflammation, it might be reasoned that inflammation persisted despite cure of MDR-TB patients.

In DS-TB patients, it was observed that as treatment progresses, lysate IL-6 reduces especially at 2 months and 6 months of drug treatment. It might be proposed that this is an indication of reduced inflammation with cure of DS-TB patients.The blood level of IL-6 was measured at one point but not at 2 months, 4 months and 6 months after the first blood collection in the control because it was previously reported that the levels of IL-6 and other cytokines do not change within such period of our study unless there are infections or environmental changes[34]

In conclusion, our results demonstrated that lysate IL-6 might be considered a potential indicator of inflammation during treatment of MDR-TB patients.

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