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In vitro QuantiFERON-TB gold antigen specific interleukin-1beta to diagnose TB among HIV-positive subjects

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SUMMARY

Background: The recently introduced IFN-γ release assay (IGRA) has been reported to improve the diagnosis of TB. However, IGRA has suboptimal sensitivity to diagnose TB among HIV co-infected subjects. Apart from IFN-γ, the pro inflammatory cytokines such as Interleukin-1beta (IL-1β), Tumor necrosis factor-alpha (TNF-α), IL-2, IL-6, IL-8 and IL-12 are also play a major role in mycobacterial infections. This study aimed to analyze these cytokines for detecting active TB among HIV sero positive subjects.

Materials and methods: We had prospectively enrolled 53 HIV positive subjects and 55 HIV-TB co-infected patients from India. IGRA was performed by using QuantiFERON TB-Gold In tube (QFT-GIT) method. TB antigen specific IL-1β, TNF-α, IL-2, IL-6, IL-8 and IL-12 levels were evaluated by ELISA in plasma harvested from QFT-GIT tubes.

Results and conclusion: The TB antigen specific IL-1β levels were significantly elevated in HIV-TB co-infected patients compared to HIV positive subjects ($p = 0.0004$). The specificity of both IL-1β (50.94%) and QFT-GIT (52.83%) remained similar in HIV positive subjects ($p = 0.24$). However, IL-1β had shown higher sensitivity (72.73%) than QFT-GIT (54.55%) to diagnose TB among HIV co-infected patients. Moreover, in culture test positive HIV-TB patients, antigen specific IL-1β exhibited sensitivity of 84.21%; whereas QFT-GIT exhibited only 57.89% sensitivity. Unlike IFN-γ (the read out marker of QFT-GIT), antigen specific IL-1β levels were not influenced by low CD4 counts. The other cytokine levels were not significantly differ between the 2 groups.From this study we concluded that TB antigen specific IL-1β may be an additional biomarker for active TB diagnosis among HIV positive subjects.

1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M. tb*), remains one of the deadliest infectious diseases of mankind. According to WHO-2014 global TB report, an estimated 9 million people developed TB and 1.5 million died from the disease in 2013 [1]. This situation is further worsened by emergence of drug resistant strains of *M. tb* [2], human immuno deficiency virus (HIV) co-infection [3] and latent TB infection (LTBI) [4]. Individuals with HIV infection are at increased risk of re-activation of LTBI; as well as of rapid progression of a recently acquired tuberculous infection [5]. An estimated 1.1 million (13%) of the 9 million people who developed TB in 2013 were HIV-positive [1]. Delay in diagnosis and in initiation of appropriate treatment for TB lead to 45–85% deaths among HIV co-infected patients [6]. Thus early diagnosis and accurate treatment of TB are the key elements to control death rate of HIV co-infected patients.

Due to non-specific clinical features, diagnosis of TB among HIV co-infected patients remains difficult. Decreased tuberculin reactivity, reduced sensitivity of acid fast staining and atypical radiographic presentations hinder the diagnosis of TB in HIV co-infected patients [7]. The recent exploration in diagnosis of TB infection is the development of Interferon-gamma (IFN-γ) release assays (IGRAs). Although IGRAs are intended for LTBI and not active TB, there is concern about increasing use of IGRAs for active TB in high-burden countries [8]. Studies from low- and middle-income

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countries had demonstrated sensitivity of 69%–83% and specificity of 52%–61% for IGRAs in the diagnosis of active TB [9]. However, IGRA has suboptimal sensitivity (60%–70%) to detect active TB among the HIV co-infected patients, suggesting that ~1 in 3 HIV-TB co-infected patients will have negative IGRA results [9]. In HIV-TB co-infected patients, the reduced sensitivity of IGRA is often associated with indeterminate results [5].

In IGRA test, antigen specific T-cells secrete a plethora of cytokines and evaluation of these secreted cytokines other than IFN-γ, may be a useful approach for diagnosis of active TB among HIV co-infected patients. Previously, we had measured 6 potential biomarkers, such as TB antigen specific interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), IL-2, IL-6 IL-8 and IL-12(p40) in 3 study groups such as TB patients (PTB), healthy household contacts (HHC) and healthy control subjects (HCS). Antigen-specific IL-1β and TNF-α levels were significantly higher in PTB than HHC and HCS. Moreover, antigen-specific IL-1β assay could differentiate between PTB and HHC; other cytokines levels did not differ among the study groups [10].

Thus the present study is extended to evaluate diagnostic ability of these 6 TB antigen specific cytokines in HIV and HIV-TB co-infected patients and to determine whether the diagnostic performance of IGRA could be improved by addition of these cytokines.

2. Materials and methods

2.1. Study subjects

The present study was approved by Institutional Ethical Committee of National Institute for Research in Tuberculosis (NIRT), Chennai. All the study subjects were informed about the study procedure and written consent was obtained from all the volunteers. Individuals with previous history of TB, those who underwent TST in the past 16 months, those with silicosis, end stage renal disease and leukemia/lymphoma were excluded from this study.

HIV positive subjects (N = 53), were recruited from Government Hospital of Thoracic Medicine (GHTM), Chennai. All the subjects were apparently free of TB symptoms and did not have any close contact with TB patients. All were having normal chest X-ray (read by 2 clinicians) and they were negative for both sputum smear and culture test for TB; but sero positive for HIV.

HIV-TB patients (N = 55), were also recruited from GHTM, Chennai. This group included subjects who were having abnormal X-ray and at least one positive sputum smear and/or culture for TB, with sero positivity for HIV who had received <1 week ATT treatment.

2.2. HIV testing

The HIV infection status was confirmed by 2 rapid tests (Retroquic Comb Aids-RS, Span Diagnostics, India and HIV TRI-DOT, J. Mitra & Co, India). When a serum was positive for both tests, it was considered as HIV positive. If a serum was positive for only one EIA, Western Blot was done as confirmatory test.

2.3. CD4 count

The CD4 cell count was estimated in blood samples of all the study subjects by flow cytometry. As described previously [5], 100 μl of whole blood was labeled with saturating concentrations of anti CD3-FITC, anti CD4-PE and anti CD8-APC (BD Biosciences, CA, USA). The percentages of CD3, CD4 and CD8 cells among the total lymphocytes were obtained using Flowjo Software (Tree star, Inc., CA, USA). The absolute CD3, CD4 and CD8 counts were calculated by multiplying the percentage with the total lymphocyte count.

2.4. IGRA

As described previously [10], IGRA was performed using QuantiFERON-TB Gold In-Tube (QFT-GIT) (Cellestis Ltd, Carnegie Victoria, Australia) kit as per manufacturer's instructions. The test results were interpreted using the software given by the manufacturer and the cut-off point for the diagnosis was determined as per the manufacturer's instructions.

2.5. Measurement of cytokines

The levels of IL-1β, TNF-α, IL-2, IL-6, IL-8 and IL-12p(40) in QFT-GIT supernatants were measured by standard ELISA technique using commercially available BD opt-EIA Kit (BD Biosciences, Franklin Lakes, NJ, USA) as per the manufacturer's instructions.

2.6. Statistical analysis

Statistical analysis was performed by using GraphPad Prism software version 5.0 (GraphPad software, CA, USA). The median value for each group was determined and compared using Mann–Whitney U test. In all instances, a $p < 0.05$ was considered as significant. Receiver-operating-characteristic (ROC) curves were used to determine the cut-off points and discriminative ability was evaluated by the area under the ROC curve (AUC). Fisher exact test was used to compare the proportion of positivity between IL-1β and QFT-GIT assays. Spearman's rank correlation coefficient was used to measure the correlation between CD4+ counts and antigen specific cytokine levels.

3. Results and discussion

Table 1 shows the demographic and baseline characteristics of the 2 study groups. Among 53 HIV and 55 HIV-TB patients, QFT-GIT had shown 7 and 14 indeterminate results respectively. In this study we had estimated the diagnostic accuracy of QFT-GIT with, and without the indeterminate results considered as negative [10–12].

Table 1
Demographic and baseline characteristics of the study groups.

	HIV	HIV-TB
No. of subjects (N)	53	55
Age	19–56	21–52
Sex		
Male	29 (55%)	38 (69%)
Female	24 (45%)	17 (31%)
HIV strain		
HIV–I	46 (87%)	51 (93%)
HIV–I &II	7 (13%)	4 (7%)
Smear test status		
Positive	–	42 (76.36%)
Negative	–	13 (23.63%)
Culture test (available for)	–	19 (34.54%)
Smear negative-culture positive, N	–	13
Smear positive-culture positive, N	–	6
Chest X-ray (available for)	–	31 (56.36%)
QFT-GIT		
Positive	25 (47.16%)	30 (54.54%)
Negative	21 (39.62%)	11 (20%)
Indeterminate	7 (13%)	14 (25.45%)
CD4+ cell count		
<100 cells/μl	12 (22.64%)	22 (40%)
100 to 200 cells/μl	14 (26.41%)	17 (30.90%)
>200 cells/μl	27 (50.94%)	16 (22.09%)

% – Percentage.