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Original Research

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Heightened Plasma Levels of Heme Oxygenase-1 and Tissue Inhibitor of Metalloproteinase-4 as Well as Elevated Peripheral Neutrophil Counts Are Associated With TB-Diabetes Comorbidity

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Background: **The increased prevalence of type 2 diabetes mellitus (T2DM) in countries endemic for TB poses a serious complication in the clinical management of this major infectious disease. Understanding the impact of T2DM on TB and the determinants of comorbidity is critical in responding to this growing public health problem with better therapeutic approaches. Here, we performed an exploratory study assessing a series of biologic parameters that could serve as markers of pathogenesis in TB with T2DM.**

Methods: **Cross-sectional analyses of levels of heme oxygenase-1 (HO-1), acute phase proteins, tissue metalloproteinases, and tissue inhibitors of metalloproteinase (TIMPs) as well as cytokines and chemokines were performed in plasma samples from individuals with active pulmonary TB or with coincident TB and T2DM from South India.**

Results: **Compared with patients with TB without diabetes, those with coincident T2DM exhibited increased *Mycobacterium tuberculosis* bacillary loads in sputum. Plasma levels of HO-1 but not of other acute phase proteins were higher in patients with TB and T2DM than in patients without diabetes, independent of bacillary sputum loads. HO-1 concentrations also positively correlated with random plasma glucose, circulating glycosylated hemoglobin, and low-density lipoprotein levels. Moreover, patients with coincident TB and T2DM exhibited increased plasma levels of TIMP-4 and elevated peripheral blood neutrophil counts, which, when considered together with HO-1, resulted in increased power to discriminate individuals with active TB with and without T2DM.**

Conclusions: **Elevated plasma levels of HO-1 and TIMP-4 and peripheral blood neutrophil counts are potential single and combined markers of pathogenesis in TB and T2DM.**

CHEST 2014; 145(6):1244–1254

Abbreviations: AFB = acid-fast bacilli; ANC = absolute neutrophil count; CRP = C-reactive protein; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; HO-1 = heme oxygenase-1; IFN = interferon; LDL = low-density lipoprotein; MMP = matrix metalloproteinase; Mtb = *Mycobacterium tuberculosis*; PCA = principal component analysis; ROC = receiver operating characteristic; SAA = serum amyloid protein-A; T2DM = type 2 diabetes mellitus; TIMP = tissue inhibitor of metalloproteinase; TNF = tumor necrosis factor

Type 2 diabetes mellitus (T2DM) and pulmonary TB are two of the most prevalent comorbid conditions in many parts of the world, and the convergence of these diseases appears to pose a serious threat to health care worldwide. Indeed, a variety of clinical and epidemiologic studies have identified T2DM as a risk factor for the development of active TB.[1] Moreover, T2DM also appears to be associated with a greater severity of TB disease among the infected population and to have a detrimental effect on both disease presentation and response to treatment.[2,3] Although the clinical and public health significance posed by the dual burden of TB and T2DM has been increasingly recognized, data examining the immunologic and metabolic

basis of susceptibility to TB in patients with diabetes remain scarce. Although enhanced susceptibility to TB in patients with T2DM was initially attributed to a relative immunodeficiency among patients with diabetes, recent experimental and clinical studies are not consistent with this explanation. Indeed, increased levels of T helper cell 1, T helper cell 17, or both cytokines have been described in both TB-infected diabetic mice[4] and humans with TB and diabetes.[5,6]

Heme oxygenase-1 (HO-1) is a major antioxidant highly expressed in lung tissue and is a key stress-response enzyme that degrades heme molecules, releasing free iron, carbon monoxide, and biliverdin.[7] Recently, we have shown that HO-1 levels can distinguish latent or successfully treated TB from active disease with high accuracy[8] and that HO-1 levels can also distinguish latent *Mycobacterium tuberculosis* (Mtb) infection from active disease in children.[9] Interestingly, HO-1 levels have also been found to be elevated in T2DM, a finding attributed to an enhanced proinflammatory environment present in these individuals.[10] Although HO-1 has been shown to be an important biomarker for TB severity, it has also been shown that other inflammatory markers, including acute phase reactants[11] and matrix metalloproteinases (MMPs) and their endogenous inhibitors,[12,13] can be used to distinguish patients with active TB.

Therefore, we hypothesized that measurement of HO-1 in combination with other markers might be used to detect increased comorbidity in TB with T2DM. To test this, we measured the levels of HO-1 and a variety of other markers in a group of pulmonary Mtb-infected individuals with and without T2DM. Our data reveal that elevated plasma levels of HO-1 and tissue inhibitor of metalloproteinase (TIMP)-4, in addition to increased absolute neutrophil counts (ANCs) in the blood, are potential markers of pathogenesis in TB with T2DM.

Manuscript received August 1, 2013; revision accepted December 17, 2013; originally published Online First January 23, 2014.
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Funding/Support: This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.
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DOI: 10.1378/chest.13-1799

Materials and Methods

Study Population

We studied a group of 88 individuals with active pulmonary TB recruited from the TB Clinic at Stanley Medical Hospital, Chennai, India—the first consecutive 44 with diabetes and the first 44 without (Table 1). All individuals were sputum smear and culture positive. T2DM was diagnosed on the basis of glycosylated hemoglobin (HbA1c) levels and random blood glucose test, according to the American Diabetes Association criteria (all individuals with T2DM had HbA1c levels > 6.5% and random plasma glucose > 200 mg/dL). All individuals tested negative for HIV and were naive to antituberculous treatment. The two groups did not differ significantly in terms of radiologic extent of disease or site of disease as assessed by chest radiograph readings from three independent experts (data not shown). Anthropometric measurements and biochemical parameters, including plasma glucose, lipid profile, and HbA1c levels, were obtained using standardized techniques detailed elsewhere.[14] Hematology was performed on all individuals using the Act-5 Diff-hematology analyzer (Beckman Coulter, Inc). The clinical databank used for the present study did not contain information on other comorbidities or medications used to treat diabetes and associated comorbid conditions. All individuals were examined as part of a clinical protocol approved by the National Institutes of Research in Tuberculosis Internal Ethical Committee (study ID: NCT01154959, project approval number NIRT-IEC: 2010002), and informed written consent was obtained from all participants.

Immunoassays

Levels of HO-1 (Enzo Life Sciences, Inc), apotransferrin and hepcidin (Uscn Life Science Inc), CD163, CD14, PD-1, interferon (IFN)-α, and IFN-β (R&D Systems, Inc) were measured using enzyme-linked immunosorbent assay kits. Levels of C-reactive protein (CRP), serum amyloid protein-A (SAA), haptoglobin, α_2-macroglobulin, and several cytokines and chemokines were determined using a multiplex enzyme-linked immunosorbent assay system (Bio-Rad Laboratories, Inc). Levels of MMP-1, MMP-7, MMP-8, MMP-9, TIMP-1, TIMP-2, TIMP-3, and TIMP-4 were measured using a Luminex kit from R&D Systems, Inc.

Data Analysis

The median values with interquartile ranges were used as measures of central tendency. Continuous variables were compared using Mann-Whitney tests with Holm adjustment for multiple comparisons. The χ^2 or Fisher tests were used to compare variables displayed as percentage. Spearman rank tests were used to assess correlations. Receiver operating characteristic (ROC) curves were used to test the power of each biomarker to distinguish TB with T2DM from TB with no T2DM cases. Multinomial logistic regression analyses adjusted for age, sex, BMI, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were performed to assess the OR of the associations between HO-1, TIMP-4, and/or ANC and TB with T2DM comorbidity. Three models of principal component analysis (PCA) were designed to assess how different combinations of candidate biomarkers contribute to the differentiation between TB with T2DM and TB without T2DM. In one model, only levels of HO-1, TIMP-4, and ANC were inputted. A second model inputted levels of several cytokines, chemokines, MMPs, and TIMPs, acute phase proteins, soluble markers of cellular activation, blood cell counts, and HO-1. A third model included all candidate biomarkers except for HO-1, TIMP-4, and ANC. An unsupervised two-way hierarchical cluster analysis (Ward method) using the same variables inputted in the different PCA models was used to specifically test