[Original Research]

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Hemostasis and Lipoprotein Indices Signify Exacerbated Lung Injury in Tuberculosis With Diabetes Comorbidity

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BACKGROUND: Exacerbated immunopathology is a frequent consequence of TB that is complicated by diabetes mellitus (DM); however, the underlying mechanisms are still poorly defined.

METHODS: In the two groups of age- and sex-matched patients with TB and DM (DM-TB) and with TB and without DM, we microscopically evaluated the areas of caseous necrosis and graded the extent of perinecrotic fibrosis in lung biopsies from the sputum smear-negative (SN) patients. We scored acid-fast bacilli in sputum smear-positive (SP) patients and compiled CT scan data from both the SN and SP patients. We compared inflammatory biomarkers and routine hematologic and biochemical parameters. Binary logistic regression analyses were applied to define the indices associated with the extent of lung injury.

RESULTS: Enlarged caseous necrotic areas with exacerbated fibrotic encapsulations were found in SN patients with DM-TB, consistent with the higher ratio of thick-walled cavities and more bacilli in the sputum from SP patients with DM-TB. Larger necrotic foci were detected in men compared with woman within the SN TB groups. Significantly higher fibrinogen and lower high-density lipoprotein cholesterol (HDL-C) were observed in SN patients with DM-TB. Regression analyses revealed that diabetes, activation of the coagulation pathway (shown by increased platelet distribution width, decreased mean platelet volume, and shortened prothrombin time), and dyslipidemia (shown by decreased low-density lipoprotein cholesterol, HDL-C, and apolipoprotein A) are risk factors for severe lung lesions in both SN and SP patients with TB.

CONCLUSIONS: Hemostasis and dyslipidemia are associated with granuloma necrosis and fibroplasia leading to exacerbated lung damage in TB, especially in patients with DM-TB.

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KEY WORDS: coagulation; diabetes; lipoprotein; lung injury; tuberculosis

ABBREVIATIONS: AFB = acid-fast bacillus; aOR1 = OR after being adjusted for sex, age, and BMI; aOR2 = OR after being adjusted for sex, age, BMI, and no DM/DM status; ApoA = apolipoprotein A; DM = diabetes mellitus; DM-TB = TB and DM; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MNGC = multinucleated giant cell; MPV = mean platelet volume; NDM-TB = TB without DM; PCT = plateletcrit; PDW = platelet distribution width; PT = prothrombin time; SN = sputum smear-negative; SP = sputum smear-positive; SPH = Shanghai Pulmonary Hospital

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TB remains a global health emergency. Up to one-third of the world's population is infected with *Mycobacterium tuberculosis*. In 2015, there were an estimated 10.4 million new (incident) TB cases and an estimated 1.8 million TB deaths worldwide.[1] To worsen the situation, the global pandemic of diabetes mellitus (DM) poses a serious threat to the prevention and therapy of TB.[2] Epidemiologic studies have revealed a threefold higher risk of TB among patients with DM than among individuals without DM and a strong association between TB and DM regardless of TB endemicity.[3] Moreover, comorbidity of patients with TB and DM and (DM-TB) results in a higher probability of treatment failure, multidrug resistance, and death.[4] Although experiments in animal models indicate that DM exacerbates TB immunopathology,[5] evidence based on human pathology samples is still scarce. Deeper understanding is urgently needed to develop novel control measures for severe TB, including DM-TB.[6]

Here, we compared the grades of lung lesions and the lung lesion-associated bacilli in sputum smears from two groups of age- and sex-matched patients with DM-TB and patients with TB without DM (NDM-TB) from early and late-stage TB, respectively. Evaluation of the biochemical and immunologic indices followed by regression analyses emphasize the association of hemostasis and lipoprotein-associated factors with the pathogenesis of TB,[7,8] especially with that of DM-TB.[9]

Materials and Methods

The design of the study is shown in e-Figure 1. Two groups of age- and sex-matched patients with DM-TB or with NDM-TB were chosen to retrospectively compare lung lesions (the area of caseous necrosis and perifibrotic encapsulation of the granuloma), sputum bacterial counts, high-resolution CT scans, and changes in/association with hematologic, biochemical, and coagulation indices.

Clinical Sample Selection and Data Collection

Tissue samples from 45 sputum smear-negative (SN) patients with NDM-TB and DM-TB (type 1 diabetes: n = 1; type 2 diabetes: n = 44) undergoing exploratory pulmonary lobectomy from January 2009 to September 2013 in Shanghai Pulmonary Hospital (SPH) were submitted to pathologic observation. TB was diagnosed according to acid-fast bacillus (AFB) staining of fluid from a percutaneous transthoracic puncture or pathologic tissue, mycobacterial culture, DNA and RNA polymerase chain reaction amplification, and histopathological microscopy, and corroborated with clinical symptoms. Hematoxylin and eosin stain and specific stains were performed to rule out a tumor (cytokeratin/CD68 stain), sarcoidosis (reticular fiber stain), and granulomas induced by etiologies other than TB (periodic acid-Schiff stain and methenamine silver stain for fungus, and Prussian blue iron stain for a foreign body granuloma).

There were 103 patients with TB confirmed by sputum smear-positive (SP) tests and cultures who were admitted to SPH from December 2012 to March 2015 who constituted the SP with NDM-TB and DM-TB (type 1 diabetes: n = 2; type 2 diabetes: n = 101) groups.

To identify inflammatory and metabolic parameters associated with the type and severity of the lung lesions, 67 SN patients with TB (NDM-TB: n = 34; DM-TB: n = 33) and 139 SP patients with TB (NDM-TB: n = 67; DM-TB: n = 72) with complete records of hematologic and biochemical analyses (46 indices for SN patients with TB and 55 indices for SP patients with TB) were chosen for regression analyses.

DM was diagnosed before registration or at SPH based on fasting plasma glucose levels (≥ 7.0 mmol/L), random blood glucose testing (≥ 11.1 mmol/L), and glycated hemoglobin (≥ 6.5%) according to the diabetes diagnostic criteria of the Chinese Diabetes Society[10] and in accordance with the World Health Organization diagnostic criteria for diabetes.[11]

All individuals tested were negative for HIV and had no history of TB treatment. None were undergoing corticosteroid therapy. Patients with history of tumor, rheumatoid arthritis, or asthma were excluded. All anthropometric, biochemical, and hematologic parameters were the first medical records acquired during patient admission.

Evaluation of Intragranulomatous Caseous Necrosis and Perinecrotic Fibroplasia

For each patient, one to five lung sections from formalin-fixed, paraffin-embedded blocks from different sites of granuloma lesions were processed. A total of 267 hematoxylin and eosin-stained sections (5 μm; NDM-TB sections: n = 129; DM-TB sections: n = 138) were evaluated by two pathologists who were blinded to the patient categories.

Histopathologic features of hematoxylin and eosin and Masson trichrome stain sections were observed under a microscope (Leica Camera AG) with a 5× objective lens (field number, 22; actual field of view, 15.2 mm^2; calculated using the formula $S = \pi \times {}^1/_4 \times [\text{field number}/5]^2$). Necrotic areas of each section were identified and recorded. Individuals with more than one biopsy section were judged as <15.2 mm^2 only when all sections were judged as <15.2 mm^2.

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