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Tuberculosis-diabetes co-morbidity is characterized by heightened systemic levels of circulating angiogenic factors

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Summary *Background:* Tuberculosis-diabetes co-morbidity (TB-DM) is characterized by increased inflammation with elevated circulating levels of inflammatory cytokines and other factors. Circulating angiogenic factors are intricately involved in the angiogenesis-inflammation nexus.
Methods: To study the association of angiogenic factors with TB-DM, we examined the systemic levels of VEGF-A, VEGF-C, VEGF-D, VEGF-R1, VEGF-R2, VEGF-R3 in individuals with either TB-DM (n = 44) or TB alone (n = 44).
Results: Circulating levels of VEGF-A, C, D, R1, R2 and R3 were significantly higher in TB-DM compared to TB individuals. Moreover, the levels of VEGF-A, C, R2 and/or R3 were significantly higher in TB-DM with bilateral or cavitary disease or with hemoptysis, suggesting an association with both disease severity and adverse clinical presentation. The levels of these factors also exhibited a significant positive relationship with bacterial burdens and HbA1c levels. In addition, VEGF-A, C and R2 levels were significantly higher (at 2 months of treatment) in culture positive compared to culture negative TB-DM individuals. Finally, the circulating levels of VEGF-A, C, D, R1, R2 and R3 were significantly reduced following successful chemotherapy at 6 months.
Conclusion: Our data demonstrate that TB-DM is associated with heightened levels of circulating angiogenic factors, possibly reflecting both dysregulated angiogenesis and exaggerated inflammation.
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Introduction

Diabetes mellitus (DM) increases the risk of pulmonary tuberculosis (TB) by 2—3 fold and is associated with greater odds of cavitary disease, positive sputum smear, delayed sputum conversion, treatment failure, relapse and death.[1,2] TB-DM co-morbidity is now a common occurrence in most low and middle income countries endemic for TB and serves as the major impediment in the elimination of TB worldwide.[3] The pathogenesis of TB-DM co-morbidity is not completely understood, but chronic inflammation appears to be the central underlying pathogenic feature.[4,5] In addition, dysregulated angiogenesis also appears to be a major characteristic of TB and DM independently.[6,7] Angiogenesis is typically regulated by the vascular endothelial growth family members, which includes VEGF-A, VEGF-B, VEGF-C and VEGF-D.[8] These factors bind with differing specificities to three mostly endothelial receptors - VEGF-R1, VEGF-R2 and VEGF-R3 to stimulate angiogenic processes.[9]

Previous studies have demonstrated that elevated levels of VEGF-A is a characteristic feature of TB and that VEGF-A serves as an important biomarker distinguishing active disease from latent infection.[7,10—14] In addition, we have recently shown that VEGF-A, VEGF-C and VEGF-R2 are all accurate biomarkers of disease severity, bacterial burden and response to treatment in pulmonary TB.[15] It has also been reported that interference with angiogenic or lymphangiogenic pathways in experimental models of mycobacterial infection results in significantly improved treatment outcomes as well as diminished mycobacterial growth.[16,17] In addition, angiogenesis plays a prominent role in the pathogenesis of DM and its complications.[6] A paradoxical feature of DM pathogenesis appears to be that vascular impairment (or diminished angiogenesis) and excessive angiogenesis can co-exist in different organs of the same host.[18]

Since angiogenesis and inflammation are intricately linked and are independent characteristics of TB and DM, we postulated that TB-DM would also be associated with heightened levels of systemic angiogenic factors. To this end, we examined the circulating levels of these angiogenic factors in individuals with TB-DM in comparison to TB alone. Our data reveal a significant elevation of all circulating angiogenic factors in TB-DM and a significant association of VEGF-A, VEGF-C and VEGF-R2 with disease severity, adverse clinical presentation, bacterial burden and poor glycemic control. Our data also suggest that the factors mentioned above could serve as accurate biomarkers for monitoring therapeutic responses in TB-DM.

Materials and methods

Ethics statement

This study was approved by the Ethics Committees of the Prof. M. Viswanathan Diabetes Research Center and NIRT. Informed consent was obtained from all participants.

Study population

Plasma samples were collected from 44 individuals with active pulmonary TB and diabetes mellitus (TB-DM) and 44 individuals with pulmonary TB and no diabetes (TB). These individuals were a subset of individuals recruited for the "Effects of Diabetes on Tuberculosis Severity" study presently underway at the Prof. M. Viswanathan Diabetes Research Center and the National Institute for Research in Tuberculosis.[19] Consecutively enrolled individuals were recruited for this study. The baseline demographic characteristics of the study population are shown in Table 1. PTB was diagnosed on the basis of sputum smear and culture positivity. Chest X-rays were used to determine cavitary disease as well as unilateral vs. bilateral involvement. Smear grades were used to determine bacterial burdens and classified as 1+, 2+ and 3+. At the time of enrollment, all active TB cases had no record of prior TB disease. DM was diagnosed on the basis of oral glucose tolerance test and/or glycated hemoglobin (HbA1c) levels (for known diabetics), according to the WHO criteria. The DM individuals were a combination of known DM (n = 34) and newly diagnosed DM (n = 10). All the individuals were HIV seronegative and anti-tuberculous treatment naïve. Anthropometric measurements, hematological and biochemical parameters were obtained using standardized techniques. All individuals had pan-sensitive *Mycobacterium tuberculosis* on sputum culture at enrollment and all received standard tuberculosis treatment (Directly Observed Treatment Short Course — DOTS with isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months, followed by isoniazid and rifampicin for 4 months). All individuals were smear and culture negative at the end of 6 months of therapy (Table 1). Blood samples were collected at baseline, 2 months and 6 months of anti-TB treatment (ATT). These PTB individuals are different from the individuals described in our previous study.[15]

ELISA

Circulating levels of VEGF-A, VEGF-C, VEGF-R1, VEGF-R2 and VEGF-R3 were measured using the Duoset ELISA Development System (R&D Systems). Quantikine ELISA kit (R&D Systems) was used for measuring VEGF-D. The lowest detection limits were as follows: VEGF-A, 31.25 pg/ml; VEGF-C, 62.5 pg/ml; VEGF-D, 62.5 pg/ml; VEGF-R1, 125 pg/ml; VEGF-R2, 31.25 pg/ml; VEGF-R3, 156.25 pg/ml.

Statistical analysis

Geometric means (GM) were used for measurements of central tendency. Statistically significant differences between the two groups were analyzed using the Mann—Whitney test with Holm's correction for multiple comparisons. Linear trend post-test was used to compare angiogenic factor concentrations with smear grades (reflecting bacterial burdens) and Spearman rank correlation was used to compare angiogenic factor concentrations with HbA1c levels. Wilcoxon signed rank test was used to compare angiogenic factor concentrations before, during and after