Published in final edited form as:

Cytokine. 2016 March ; 79: 74–81. doi:10.1016/j.cyto.2015.12.026.

Type 2 diabetes mellitus coincident with pulmonary or latent tuberculosis results in modulation of adipocytokines

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Abstract

Type 2 diabetes mellitus (T2DM) is recognized as major risk factor for the progress of active pulmonary tuberculosis (PTB), although the mechanistic link between diabetes and tuberculosis remains poorly characterized. Moreover, the influence of poorly controlled diabetes on the baseline levels of adipocytokines in the context of tuberculosis has not been explored in detail. To characterize the influence of coexistent DM on adipocytokine levels in pulmonary or latent TB (LTB), we examined circulating levels of adipocytokines in the plasma of individuals with PTB-DM or LTB-DM and compared them with those without DM (PTB or LTB). PTB-DM or LTB-DM is characterized by diminished circulating levels of adiponectin and adipisin and/or heightened circulating levels of leptin, visfatin and PAI-1. In addition, adiponectin and adipisin exhibit a significant negative correlation, whereas with leptin, visfatin and PAI-1 display a significant positive correlation with HbA1C levels and random blood glucose levels. Therefore, our data reveal that PTB-DM or LTB-DM is characterized by alterations in the systemic levels of adipocytokines, indicating that altered adipose tissue inflammation underlying Type 2 diabetes potentially contributes to pathogenesis of TB disease.

Keywords

Mycobacterium tuberculosis; Diabetes Mellitus; Adipocytokines; Immune response; Immune regulation

Diabetes mellitus (DM) has been documented as a risk factor for tuberculosis (TB) for decades. Although the association between Type 2 diabetes mellitus (T2DM) and TB is not new, only with the current explosive DM pandemic has the prominence of understanding the associations between T2DM and TB arisen as a global health priority [1]. Preceding reports

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found that patients with DM were two to eight times at advanced risk for development of active TB as compared with patients without DM [1-3].

The term adipocytokine is used to define cytokines that are mostly produced by adipose tissue. The adipocytokines, adiponectin and leptin have been described as the most important adipocyte products, thereby redefining adipose tissue as a main element not only of the endocrine system, but also of the immune system [4]. Adipose tissue is an important inflammatory source in obesity and type 2 diabetes, not only because of cytokines produced from the adipocyte itself, but also because of infiltration by proinflammatory macrophages [5, 6]. In humans, adipocytokines function as hormones to control energy homeostasis and to stabilize endocrine function. As cytokines, they influence immune functions and inflammatory processes throughout the body [4]. Adiponectin, leptin, resistin and visfatin are adipocytokines thought to deliver an important link between insulin resistance and related inflammatory disorders [7-11]. Adiponectin and leptin, function in a hormone-like manner and have many of the feature of conventional cytokines and there is escalating evidence that they are elaborated in many diseases and, under certain circumstances, might cross regulate each other [4]. Studies have also shown that leptin is an immune system regulator in supplement to its effect on food intake [12, 13]. Plasma levels of leptin can be altered in disease states related to malnutrition [14]. Other adipocytokines, such as resistin and visfatin, are also created by adipocytes but can also be synthesized outside the adipose tissue, in specific by monocytes and macrophages [4]. Adipsin was recognized as a proinflammatory product of adipose tissue that is induced in models of diabetes and obesity, providing indication for a functional link between obesity and inflammation [15] and plasminogen activator inhibitor 1 (PAI-1), an inhibitor of fibrinolysis, as an adipokine that is strongly upregulated in visceral adipose depots in metabolic diseases [16].

Low plasma leptin and high adiponectin levels are linked with wasting and inflammation in pulmonary TB [40-41]. In addition, resistin is considered to be a surrogate biomarker for pulmonary TB while PAI-1 is known to be present at higher concentrations in pulmonary TB [42-43]. The role of adipsin and visfatin has never been investigated in TB infection or disease. Since these adipocytokines are known to affect immunity to infections, we hypothesized that a mechanism by which DM modulates TB infection or disease would be altering the systemic levels of these factors. To study influence of T2DM on active pulmonary and latent TB, we examined circulating levels of adipocytokines in the plasma of individuals with PTB-DM or LTB-DM and compared them with those without DM (PTB or LTB). We show that those with PTB-DM or LTB DM have decreased systemic levels of adiponectin and adipsin and/or increased systemic levels of leptin, visfatin and PAI-1. Thus, our data suggest that T2DM is associated with modulation of systemic adipocytokine levels in both active and latent TB.

MATERIALS AND METHODS

Ethics statement

All study participants were studied as part of a clinical protocol approved by the Institutional Review Board of the National Institute of Research in Tuberculosis (NCT01154959), and informed written consent was obtained from all the study participants.

Study Population

We studied a group of 88 individuals with active pulmonary TB from individuals attending the TB clinic of Stanley Medical Hospital, Chennai — 44 of whom with diabetes (*hereafter* PTB-DM) and 44 of whom without diabetes (*hereafter* PTB). Another set of 88 individuals with latent TB 44 of whom with diabetes (*hereafter* LTB-DM) and 44 of whom without diabetes (*hereafter* LTB). The demographics of the study population is shown in Table 1.

Individuals with pulmonary TB were diagnosed on the basis of positive clinical symptoms, positive radiological results on chest X-ray and categorized as unilateral and bilateral disease, sputum smear positivity for acid fast bacillus (AFB) by Ziehl Neelsen staining, smear grades were determined by sputum microscopy and graded as 0, 1+, 2+, and 3+ with zero being no bacteria in microscopy and 3+ the maximum number of bacteria and positive culture for *Mycobacterium tuberculosis*(Mtb) on Lowenstein–Jensen medium confirm the diagnosis of TB disease. Latent infection was diagnosed on the basis of positive results of the Mantoux skin test (induration diameter >12 mm) using 2 tuberculin units of purified protein derivative (Statens Serum Institute). Individuals were diagnosed as having LTB also on the basis of being positive in the Quantiferon-TB Gold in Tube (Cellestis) assay that measures the release of interferon (IFN) gamma after stimulation in vitro by *M.tuberculosis* antigen such as ESAT-6, CFP-10 and TB7.7. The result is described as quantification of IFN γ in international units (IU) per mL. An individual is measured positive for Mtb infection if the IFN γ response to TB antigens is above the test cut-off (after subtracting the background IFN γ response in the negative control) but having an absence of pulmonary symptoms coexisting with a normal chest radiograph.

T2DM was diagnosed on the basis of glycated hemoglobin (HbA1c) levels and random blood glucose, according to the American Diabetes Association criteria (all T2DM individuals had HbA1c levels > 6.5% and random blood glucose > 200mg/dl). All the individuals were HIV negative. The two PTB groups did not differ significantly in terms of radiological extent of disease as assessed by chest X-ray readings from three independent experts. The two PTB groups did differ significantly in smear grades with the T2DM group having worse smear grades as assessed by the Fisher's exact probability test, indicating higher bacillary burdens. All individuals were anti-tuberculous treatment naïve.

Anthropometric measurements, including height and weight, and biochemical parameters, including plasma glucose and HbA1c were obtained using standardized techniques as detailed elsewhere [17]. The four groups did not differ significantly in age, sex or BMI. This study comprised of a separate set of individuals compared to our previous study on TB-DM [18].

ELISA

Plasma was collected in Heparin Sodium tubes and after centrifugation stored at -80°C . Bioplex multiplex cytokine assay system (Bio-Rad, Hercules, CA) were used to measure the adipocytokines levels. The parameters analyzed were adiponectin, adipisin, leptin, resistin, visfatin and PAI-1. All assays were run in duplicates. The lower limit of detection were: adiponectin, 3654 pg/ml; adipisin, 1496 pg/ml; leptin, 1476 pg/ml; resistin, 197 pg/ml; visfatin, 10628 pg/ml and PAI-1, 552 pg/ml.

Statistical Analysis

For the measurements of central tendency, Geometric means (GM) were used. Statistically significant differences between two groups were examined by the nonparametric Mann-Whitney U test with Holm's correction for multiple comparisons. Correlations were calculated using Spearman rank correlation. Multivariate linear models were built to test the association between the cytokine levels and the independent variables including age, gender and BMI. GraphPad PRISM Version 5.01 or R Version 2.15.2 were used for all the statistical analysis.

RESULTS

Modulation of adipocytokines in TB disease

We measured the circulating levels of adipocytokines (adiponectin, adipsin, leptin, resistin, visfatin, PAI-1) in active pulmonary tuberculosis compared them with latent tuberculosis patients (both without diabetes). As shown in Table 2, PTB individuals revealed significantly hightented levels of resistin and PAI-1 and significantly diminished levels of adipsin, leptin and visfatin in comparison to LTB individuals. In addition, we also measured the circulating levels of these adipocytokines in PTB-DM and compared them with LTB-DM. As shown in Table 2, PTB-DM individuals also revealed significantly higher levels of resistin and PAI-1 and significantly diminished levels of adipsin, leptin and visfatin in comparison to LTB-DM. No significant changes in the circulating levels of adiponectin was observed between the different groups compared. Interestingly, all the four groups did not differ significantly in the BMI, indicating that changes in the adipocytokine levels were not a reflection of altered BMI in PTB or LTB with or without DM. Finally, we performed multivariate linear regression analysis and observed that the effect of DM on adipocytokines in TB is significant even after accounting for the influence of age, gender and BMI (data not shown).

PTB with diabetes is associated with alterations in circulating levels of adipocytokines

To characterize the impact of DM on adipocytokines in active PTB, we measured the circulating levels of adiponectin, adipsin, leptin, resistin, visfatin, PAI-1 in PTB-DM and PTB individuals (Figure 1). As shown in Fig. 1, the systemic levels of adiponectin (Geometric Mean of 70.57 ng/ml in PTB-DM versus 125.2 ng/ml in PTB) and adipsin (GM of 2.31 ng/ml vs. 3.49 ng/ml) were significantly lower in PTB-DM compared to PTB individuals. In contrast, leptin (GM of 1.54 ng/ml vs. 0.718 ng/ml) was found to be present at significantly hightented levels in PTB-DM compared to PTB individuals. Thus, PTB-DM is associated with modulation of adipocytokines in active pulmonary TB.

LTB with diabetes is associated with alterations in circulating levels of adipocytokines

To characterize the impact of DM on adipocytokines in latent TB, we measured the circulating levels of adiponectin, adipsin, leptin, resistin, visfatin, PAI-1 in LTB-DM and LTB individuals (Figure 2). As shown in Fig. 2, the systemic levels of adiponectin (Geometric Mean of 60.56 ng/ml in LTB-DM versus 98.21 ng/ml in LTB) and adipsin (GM of 3.31 ng/ml vs. 6.23 ng/ml) were significantly lower in LTB-DM compared to LTB

individuals. In contrast, leptin (GM of 12.42 ng/ml vs. 6.58 ng/ml), visfatin (GM of 52.33 ng/ml vs. 43.15 ng/ml) and PAI-1 (GM of 33.59 ng/ml vs. 25.15 ng/ml) were found to be present at significantly hightented levels in LTB-DM compared to LTB individuals. Thus, LTB-DM is associated with modulation in adipocytokines in latent TB.

Relationship between systemic adipocytokines and HbA1c levels

HbA1c is an precise indicator of the level of diabetic control and higher values reflect poor control. Thus, to study the association between the systemic levels of adipocytokines, with the grade of diabetic control, we evaluated the association of adiponectin, adpsin, leptin, resistin visfatin and PAI-1 with HbA1C levels (in %) in all the active TB and latent TB individuals in the study. As shown in Fig. 3A and 3B, the systemic levels of adiponectin and adipsin each exhibited a significant negative correlation, whereas leptin (as well as visfatin and PAI-1 in LTB) exhibited significant positive association with the HbA1c levels in the active or latent TB individuals.

Relationship between systemic adipocytokines and random blood glucose levels

Random blood glucose is an indicator of the level of diabetic control and higher values reflect poor control. Thus, to study the association between the systemic levels of adipocytokines with the grade of diabetic control, we evaluated the association of adiponectin, adpsin, leptin, resistin visfatin and PAI-1 with random blood glucose (in mg/ml) in all the active TB and latent TB individuals in the study. As shown in Fig. 4A and 4B, the systemic levels of adiponectin and adipsin each exhibited a significant negative correlation, whereas leptin (as well as visfatin and PAI-1 in LTB) exhibited significant positive association with the random blood glucose levels in the active or latent TB individuals.

DISCUSSION

The link between diabetes mellitus (DM) and tuberculosis (TB) has been documented for centuries. It has been reported that diabetic patients have a significantly advanced risk of TB than the general population [19, 20]. The global burden of DM is predictable to rise from an estimated 180 million prevalent cases currently recorded to a predicted 366 million by 2030 and the utmost increase is projected for developing countries, where TB is highly endemic [21]. The controlling of TB is highly challenging when a patient presents with DM as a comorbidity [1]. DM has also been allied with higher mortality rates compared to TB only patients; an bigger risk of TB treatment failure or relapse, and diminished 2 and 6-month culture conversion rates [22]. In fact, a latest study in Chennai, has confirmed that the prevalence of type 2 diabetes in tuberculosis patients attending out-patient clinics is approximately 25% and another 25% of these patients are pre-diabetic [23]. The immunological basis for this susceptibility to tuberculosis among those with DM is incompletely explored.

We evaluated the adipocytokines levels in pulmonary TB and latent TB individuals with coincident diabetes mellitus. Our study of the homeostatic levels of adiponectin and adipsin shows greatly reduced plasma levels of these cytokines during DM in pulmonary TB and

latent TB patients. Our data also recommend that it is poor glycemic control that may be associated with diminished systemic levels of adiponectin and adipsin. Adiponectin as a modulator of inflammation in a variety of diseases has recently been highlighted [24]. For example, in critically ill patients, adiponectin levels seems to be rapidly inhibited at the early phase and then progressively raised at the recovery phase [25]. Circulating adiponectin levels are positively correlated with insulin sensitivity assessed by using diverse insulin sensitivity techniques [25]. Adiponectin gene expression and circulating adiponectin levels are lesser in patients with type 2 diabetes than in non-diabetic individuals [25]. In animal models, mice over expressing adiponectin showed regulated glucose and partial improvement of insulin resistance and diabetes and a decrease in macrophage infiltration in adipose tissue and systemic inflammation [26]. Adipsin was recognized as a proinflammatory product of adipose tissue that is induced in models of diabetes and obesity, providing evidence for a functional link between obesity and inflammation [15]. A recent study shown that measurement of adipsin levels may be used from a diagnostic standpoint to identify those patients at high risk of developing β cell failure and accelerated diabetes [15]. T2DM patients may be risk stratified based on their adipsin level, and those with the lowest adipsin levels may deserve closer follow up compared to other glycemic controlled patients [27]. In animal models mice, lacking adipsin display worsened glucose homeostasis when placed under the metabolic stress of diet-induced obesity [28]. Therefore, our study reveals three features in terms of the effect of TB-DM comorbidity on adiponectin and adipsin. One, TB per se influences the systemic levels of adipsin but not adiponectin independent of DM. Two, modulation of adiponectin and adipsin is a prominent feature of DM in both PTB and LTB individuals. Three, unlike the other adipocytokines examined, adiponectin and adipsin are both present at diminished levels in the circulation in the presence of DM.

Our results also demonstrate alterations in other adipocytokines like leptin, resistin, visfatin and PAI-1 in pulmonary TB and latent TB patients with coincident diabetes mellitus. Our study demonstrates that the systemic levels of leptin were significantly enhanced during DM in pulmonary TB or latent TB patients. In contrast, other adipocytokines like visfatin and PAI-1 were significantly enhanced during diabetes in latent TB individuals alone. Leptin is an adipocytokine, mainly involved in modulating the immune responses and inflammation [9]. However, in contrast to adiponectin, leptin is considered to be a pro-inflammatory cytokine and it has structural similarity to other pro-inflammatory cytokines such as IL-6, IL-12 and granulocyte colony stimulating factor [29]. One potential mechanism for increased leptin levels in pulmonary or latent TB patients with diabetes could be due to the pro-inflammatory milieu that is established in both chronic states [30, 31]. Studies have also shown that leptin can modulate the immune response and favour a Th1 response while inhibiting secretion of Th2 cytokines [29, 32]. A study also suggests that patients with pulmonary tuberculosis exhibited increased leptin levels correlating with increased TNF α levels [33]. It has also been reported that leptin levels are associated with loss of appetite in pulmonary tuberculosis and that plasma leptin levels are significantly suppressed in patients with active pulmonary tuberculosis [14, 34], which is reflected in our data on PTB and LTB alone individuals as well (Table 2). The other adipocytokines like visfatin are also secreted by adipocytes in visceral fat and decrease insulin resistance [35]. It has been stated that visfatin expression correlates with visceral adiposity in humans and it also regulates glucose

levels in mice models [35]. Increased circulating levels of visfatin are also found in patients with obesity and type 2 diabetes [36, 37]. PAI-1 is a potent inhibitor of fibrinolytic pathway and in human studies, PAI-1 is found to be increased in obesity and type 2 diabetes subjects [38]. Resistin has a proinflammatory effect has been implicated in the pathogenesis of obesity and type 2 diabetes mellitus in mouse models and humans [38, 39] and correlated with insulin resistance in diabetic patients [38]. Our data on adipocytokines like visfatin and PAI-1 in LTB-DM individuals indicates that alteration of adipocytokines is an important feature associated with the immune responses to TB infection. Our data reveal that at homeostasis PTB-DM or LTB DM individuals exhibit significantly lower frequencies of adiponectin and adipsin which show a significantly negative correlation with HbA1c and random blood glucose levels, whereas other adipocytokines like leptin, visfatin and PAI-1 were significantly elevated in PTB-DM or LTB DM individuals and exhibit a significantly positive correlation. This correlation analysis clearly specifies that the modulation of adipocytokine in TB infection and disease is associated with the pathogenesis of TB disease in DM, although the exact mechanism is not known. Moreover, as shown in Table 1, there are several significant differences in the levels of adipocytokines between PTB and LTB individuals, indicating that TB infection and disease per se has an additional effect on the systemic levels of these cytokines (apart from the effect of DM control).

Our study suffers from several weaknesses. We could not correct for all possible confounding variables, including fat mass, level of inflammation and similar parameters. In addition, being a cross-sectional study, we could not draw any inferences on cause and effect. Nevertheless, our study shows that metabolic dysfunction due to imbalance in the expression of pro- and anti-inflammatory adipocytokines is an important feature that is associated with the development of TB pathogenesis. While adipocytokines may function as regulators of body homeostasis based on the nutritional status, our data suggests that modulation of these factors by DM on both active and latent TB is essentially the same and is independent of BMI. This would suggest that nutritional effects exerted by active disease is not a major player in this TB-DM comorbidity involving both LTB and PTB. Thus, further explanation of the functions and mechanisms of adipocytokines will lead to a improved understanding of the pathogenesis of metabolic disorders associated with TB. This study provides novel evidence of a relationship between the active pulmonary TB or latent TB and adipocytokines. Modulation of adipocytokines by TB infection per se independent of age, gender, BMI or DM status would suggest that chronic infections could directly regulate the secretion of these hormonal factors and indicate that the cross-talk between the metabolic, endocrine and immune status is an important player in host defense against bacterial infections. Our study also provides an impetus to perform longitudinal studies examining the role of metabolic biomarkers in the development of TB in diabetes patients, especially the roles of adipocytokines as predictors of diabetes influence on TB.

Acknowledgments

We thank the staff of Department of Clinical Research and the Department of Social Work, NIRT especially Ms Kalaiselvi and Government Stanley Hospital, Chennai, for valuable assistance in recruiting the patients for this study, R. Anuradha, V. Gopinath and Jovvian George of the NIH-ICER for technical assistance

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Highlights

- Our study of the homeostatic levels of adiponectin and adiponin reveals profoundly diminished plasma levels of these cytokines during DM in pulmonary TB and latent TB patients.
- This study provides novel evidence of a relationship between the active pulmonary TB or latent TB and adipocytokines
- Our study also provides an impetus to perform longitudinal studies examining the role of metabolic biomarkers in the development of TB in diabetes patients, especially the roles of adipocytokines as predictors of diabetes influence on TB.

Figure 1.

Altered systemic levels of adipocytokines in PTB-DM. The plasma levels of adiponectin, adipsin, leptin, resistin, visfatin, PAI-1 were measured by ELISA in PTB-DM (n=44) and PTB (n=44) individuals. The data are represented as scatter plots with each circle representing a single individual and the line representing the geometric mean. P values were calculated using the Mann-Whitney U test.

Figure 2.

Altered systemic levels of adipocytokines in LTB-DM. The plasma levels of adiponectin, adipsin, leptin, resistin, visfatin, PAI-1 were measured by ELISA in LTB-DM (n=44) and LTB (n=44) individuals. The data are represented as scatter plots with each circle representing a single individual and the line representing the geometric mean. P values were calculated using the Mann-Whitney U test.

Figure 3.

Relationship between adipocytokines and HbA1c levels. (A) Correlation between adipocytokines and HbA1c levels in active PTB individuals. (B) Correlation between adipocytokines and HbA1c levels in LTB individuals. The relationship between the plasma levels of adiponectin, adipsin, leptin, resistin, visfatin, and PAI-1 and glycemic status was examined in PTB (n=88) and LTB (n=88) individuals. The data are represented as scatter plots with each circle representing a single individual and the line represents the linear curve fit. P values were calculated using the Spearman rank correlation.

Figure 4.

Relationship between adipocytokines and random blood glucose levels. (A) Correlation between adipocytokines and random blood glucose levels in active PTB individuals (B) Correlation between adipocytokines and random blood glucose levels in LTB individuals. The relationship between the plasma levels of adiponectin, adipsin, leptin, resistin, visfatin, and PAI-1 and glycemic status was examined in PTB (n=88) and LTB (n=88) individuals. The data are represented as scatter plots with each circle representing a single individual and the line represents the linear curve fit. P values were calculated using the Spearman rank correlation.

Table 1
Study demographics

Study Demographics	PTB-DM	PTB	LTB-DM	LTB
No. subjects recruited	44	44	44	44
Age (yrs)	49 (40 - 58)	45 (40 - 55)	45 (28 - 65)	44 (29 - 60)
Sex M / F	31 / 13	30 / 14	30 / 14	29 / 15
BMI (Kg /m2)	23.90 (19.56 – 33.38)	22.16 (19.01 - 31.22)	24.65 (19.21- 33.23)	24.32 (18.55- 32.13)
HbA1c(%)	11.2 (7.54 - 14.78)	5.33 (4.46 - 6.12)	8.92 (6.53 - 13.13)	5.1 (4.57 – 5.36)
Random glucose (mg/dl)	280.4 (200 - 587)	95.5 (76 - 177)	234 (205 – 537)	94 (64 – 139)
Mantoux Skin test Positive >12mm	NA	NA	>12 mm	>12 mm
Interferon gamma release assay	NA	NA	Positive	Positive

PTB: Pulmonary TB; LTB: Latent TB; DM: Diabetes mellitus. The values represent geometric means and range (except for age where median and range are shown)

Table 2
Systemic levels of adipocytokines in pulmonary TB and latent TB individuals with or without diabetes

Adipocytokines	PTB	LTB	pValue	PTB DM	LTB DM	pValue
Adiponectin	125.2 (37.2 - 446.6)	98.2 (32.5 - 432.1)	NS	70.5 (16.1 - 749.7)	60.5 (11.2 - 446.2)	NS
Adipsin	3.4 (0.86 - 26.9)	6.1 (0.49 - 86.7)	p=0.0281	2.3 (0.35 - 14.3)	3.3 (0.71 - 13.1)	p=0.0152
Leptin	0.71 (0.25 - 3.7)	6.5 (2 - 21.2)	p<0.0001	1.5 (0.31 - 27.5)	12.4 (3.16 - 49.03)	p<0.0001
Resistin	26 (10.1 - 676)	21.6 (3.5 - 17.2)	p<0.0001	31.4 (0.053 - 337.5)	6.1 (3.2 - 14.5)	p<0.0001
Visfatin	17.1 (5.5 - 42.6)	43.1 (21.6 - 88.2)	p<0.0001	17 (2.5 - 78.8)	52.3 (30.02 - 92.3)	p<0.0001
PAI-1	93.6 (9.9 - 270.2)	25.2 (13.8 - 54.8)	p<0.0001	86.1 (3.8 - 526.6)	33.5 (15.8 - 62.2)	p<0.0001

Values represent Geometric Means (+/- 95% Confidence intervals). p values were calculated by the Mann-Whitney U test.