Slow radiological improvement and persistent low-grade inflammation after chemotherapy in tuberculosis patients with type 2 diabetes

Akhirunnesa Mily
Karolinska Institutet
Protim Sarker
International Centre for Diarrhoeal Disease Research Bangladesh
Inin Taznin
International Centre for Diarrhoeal Disease Research Bangladesh
Md. Delwar Hossain
Bangladesh Institute of Research and Rehabilitation in Diabetes Endocrines and Metabolic Disorders
Md. Ahsanul Haq
International Centre for Diarrhoeal Disease Research Bangladesh
SM. Mostofa Kamal
National Institute of the diseases of the Chest and Hospital, Mohakhali
Birgitta Agerberth
Karolinska Institutet
Susanna Brighenti
Karolinska Institutet
Rubhana Raqib (rubhana@icddrb.org)
International Centre for Diarrhoeal Disease Research

Research article

Keywords: Anti-inflammatory cytokine, Diabetes mellitus, IL-10, Pulmonary pathology, Sputum culture, TB score

Posted Date: September 16th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23938/v2

Version of Record: A version of this preprint was published on December 7th, 2020. See the published version at https://doi.org/10.1186/s12879-020-05473-x.

Abstract

Background: Diabetes mellitus type 2 (DM) may impede immune responses in tuberculosis (TB) and thus contribute to enhanced disease severity. In this study, we aimed to evaluate DM-mediated alterations in clinical, radiological and immunological outcomes of TB disease.

Methods: Newly diagnosed pulmonary TB patients with or without DM (TB n=40; TB-DM n=40) were recruited in Dhaka, Bangladesh. Clinical symptoms, sputum smear and culture conversion as well as chest radiography were assessed, and peripheral blood and sputum samples were collected at the time of diagnosis (baseline) and after 1, 2 and 6 months of standard anti-TB treatment. Blood samples were also obtained from the healthy controls (n=20). mRNA expression of inflammatory markers in blood and sputum samples were quantified using real-time PCR.

Results: Majority of TB-DM patients had a poor glycemic control (HbA1c>8%) and displayed elevated pulmonary pathology (P=0.039) particularly in the middle (P<0.004) and lower lung zones (P<0.02) throughout the treatment period. However, reduction of clinical symptoms, and time to sputum smear and culture conversion did not differ between the groups. Transcripts levels of pro-inflammatory cytokines IL-1β (P=0.003 at month-1 and P=0.045 at month-2) and TNF-α (P=0.005 at month-1) and the anti-inflammatory cytokine IL-10 (P=0.005 at month-2) were higher in peripheral blood after anti-TB treatment in TB-DM compared to TB patients. Instead, in sputum, TB-DM patients showed reduced CD4 (P<0.009 at month-1) and IL-10 (P=0.005 at month-1 and P=0.006 at month-2) and elevated CD8 (P=0.016 at month-2) transcripts. At 1- and 2-months post-treatment, sputum IL-10 transcripts were inversely correlated with fasting blood glucose and HbA1C levels in all patients.

Conclusion: Insufficient up-regulation of IL-10 in the lung may fuel persistent local inflammation promoting lung pathology in TB-DM patients with poorly controlled DM.

Background

The convergence of tuberculosis (TB) and type 2 diabetes mellitus (DM) has emerged as a serious threat to global TB control. Type 2 DM patients is estimated to have a 3-fold higher risk of developing active TB infection compared to non-diabetic individuals [1]. Moreover, it is predicted that by 2030, about 80% of all type 2 DM patients will be living in developing countries that also have a high incidence of TB [2]. In the WHO Global report 2019, Bangladesh is ranked seventh among high-burden countries for TB, accounting for 4% of global TB cases. At the same time, the prevalence of DM is dramatically increasing in Bangladesh; the prevalence was about 7% among adults in 2017 according to the International Diabetes Federation. TB is a chronic infection caused by *Mycobacterium tuberculosis* (Mtb), while DM is considered a non-communicable, metabolic disease. Poor glycemic control is considered a major risk factor for the development of TB in DM patients [3] and may contribute to alterations in peripheral as well as local immune cell responses at the site of Mtb infection.

Several studies have demonstrated that diabetic TB patients are more often sputum positive, than TB patients without DM at the end of 2 months intensive phase anti-TB treatment [4–8]. However, other studies failed to detect differences in sputum smear or culture conversion between TB-DM and TB patients [9, 10]. TB-DM co-morbidity at the time of TB diagnosis has frequently been associated with a hyper-inflammatory cytokine profile [11, 12], and a plasma biomarker assay recently demonstrated that this systemic inflammatory state persisted in TB-DM patients throughout effective anti-TB treatment [13]. Excess systemic circulation of pro-inflammatory cytokines including IL-8, IL-12p70, and TNF-α and reduced anti-inflammatory cytokines such as IL-10, could promote non-resolving inflammation in TB-DM patients with unfavorable effects on TB disease progression and anti-TB treatment outcomes [13]. While TNF-α and IL-1β stimulates antimicrobial and other protective effects in immune cells, prolonged and excess expression of these cytokines can result in overt pathology and tissue damage [14, 15]. IL-10 counteracts these effects by inhibiting the generation of pro-inflammatory mediators in immune cells [16]. However, Mtb may also exploit the regulatory function of IL-10 to suppress imperative immune cell responses [17, 18]. As such, persistently higher bacillary burden and more severe TB disease in DM patients have been suggested to be attributed to delayed adaptive immune responses to Mtb in the lung or lung-draining lymph nodes [19, 20]. Impaired T helper 1 (Th1) responses, and lower Th1-to-Th2 cytokine ratio in peripheral blood, could result in a Th2-biased immune response in TB-DM patients [21–23]. Moreover, the cytotoxic activity of CD8 + T cells has been shown to be altered in patients with TB-DM comorbidity [24, 25]. Overall, there is no scientific consensus with regards to the role of DM in the progression of TB disease as several of these immunological changes may occur without loss of antimicrobial activity or reduction of bacterial loads [26–28].

We aimed to evaluate the effects of DM on TB-associated local and systemic inflammation as well as clinical and radiological manifestation of TB disease at baseline and after start of standard anti-TB treatment.

Methods

Patients and treatment

This observational study was conducted in two tertiary care hospitals in Dhaka from June 2014 to May 2017. TB patients (n = 40) were recruited from the National Institute of the Diseases of the Chest and Hospital (NIDCH) and TB-DM patients (n = 40) from Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM). Age- and sex-matched healthy individuals (n = 20) were randomly recruited as controls. Diabetes status was defined (WHO criteria) as patients having either a fasting blood glucose concentration ≥ 7 mmol/liter or HbA1c ≥ 6.5%. Inclusion criteria: adult males and females, age 18–60 years, newly diagnosed TB using sputum-smear microscopy and/or GeneXpert MTB/RIF and a history of diabetes for ≤ 5 years (TB-DM group). Exclusion criteria: previous history of TB, systemic or miliary TB, > 1 week of anti-TB treatment, pregnancy, history of type 2 DM for ≥ 9 years, and concomitant illnesses such as cardiovascular, liver or kidney diseases, cancer or HIV infection.

Standard anti-TB treatment involved directly observed therapy short-course (DOTS) regimen consisting of isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months followed by isoniazid and rifampicin for subsequent 4 months. At enrollment, DM patients received different types of anti-diabetic medications including metformin hydrochloride (n = 3), insulin (n = 29), a combination of metformin and insulin (n = 7) or other drugs (n = 1).

Clinical Samples And Procedures

After enrolment, socio-economic status (SES), body weight, height, clinical history including duration of illness, history of contact with active TB cases, *Bacillus Calmette–Guérin* (BCG) vaccination, smoking habits and medication history (TB-DM patients) were recorded. SES was estimated utilizing a wealth index, generated through principal component analysis of household assets [29]. At baseline, and months- 1, -2 and − 6 after initiation of standard anti-TB treatment, clinical evaluation (mentioned in Table 1 and Table S1) and radiological examinations were performed.

Table 1
Baseline characteristic of the study participants

Variables	TB (n = 40)	TB-DM (n = 40)	*p*-value[a]	Healty Controls (n = 20)	*p*-value[b]	*p*-value[c]
Sex, Male (n, %)	29 (75.5)	39 (97.5)	0.0024	14 (70.0)	0.466	< 0.001
Age (years)	26.6 ± 7.6	40.1 ± 8.8	< 0.0001	32.5 ± 6.3	0.036	0.030
BMI (Kg/m2)	17.6 ± 2.7	21.7 ± 2.5	< 0.001	26.1 ± 3.9	< 0.001	< 0.005
Family SES						
1st tertile (poor), n (%)	19 (47.5)	8 (20.00)		0		
2nd tertile (middle), n (%)	13 (32.5)	15 (37.5)		3		
3rd tertile (rich), n (%)	8 (20.00)	17 (42.5)		17		
BCG vaccination status, n (%)	23 (57.5)	35 (87.5)	< 0.0025	18 (90.0)	0.023	0.775
History of contact with active cases	19 (54.3%)	16 (45.7%)	0.255	-		
Duration of Symptom (days)	61(30, 105.5)	75.5(60, 121)	0.087	-		
Sputum smear results (AFB), n (%)						
[d]AFB negative	0	2 (5%)		-		
1 + AFB	9 (22.5%)	10 (25.0%)		-		
2 + AFB	11 (27.5%)	9 (22.5%)		-		
3 + AFB	20 (50.0%)	19 (47.5%)		-		

Quantitative data are presented as median ± IQR; Categorical data are presented as n (%). Statistical analysis comparing [a]TB vs TB-DM, [b]TB vs HC, and [c]TB-DM vs HC was done using Chi-square, Kruskal-Wallis and Dunn´s post-test or the Mann-Whitney U-test. AFB: Acid-Fast Bacilli; BCG: *Bacillus Calmette–Guérin;* BMI: body mass index; SES: socioeconomic status. [d]Sputum samples positive in the GeneXpert MTB/RIF test

Fasting blood and sputum samples were collected from the patients at baseline and at follow up visits. Healthy controls provided a fasting blood sample once. Blood glucose and HbA1c level were measured using Clinical Chemistry Analyzer (Cobas C311, Roch Diagnostics GmbH, Mannheim, Germany). Whole

blood was routinely analyzed for erythrocyte sedimentation rate (ESR) using ESR analyser (SRS 100/II, Greiner Bio-One GmbH, Kremsmunster, Austria) and complete blood count (CBC) using automated hematology analyzer (XN-1000, Sysmex Corporation, Kobe, Japan). CBC assessment included total and differential blood cell counts (Table S2) and hemoglobin concentration. Peripheral blood mononuclear cells (PBMC) and plasma were isolated from heparinized blood using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) density gradient centrifugation. Plasma levels of metabolic hormones insulin and C-peptide were measured by Luminex assay using Bio-Plex Diabetes kit (Bio-Rad Laboratories, Inc. USA). Sputum samples were used for sputum-smear microscopy and culture. PBMC and one part of sputum were stored at -80 °C in 1 ml of RNA*later* (Qiagen GmbH, Hilden, Germany) for mRNA analysis.

Clinical Composite Tb Score

A modified clinical composite TB score was determined for patients based on the presence of typical TB symptoms: fever, cough, chest pain, night sweats, hemoptysis, anemia, anorexia, weight loss and severity of lung pathology. Symptoms were recorded and scored as present (1) or absent (0) as previously described [30]. Lung involvement was scored based on the tertile values; 1st tertile score: 1, 2nd tertile score: 2 and the highest tertile score: 3. According to this scoring system, the highest expected score was 11.

Mtb Sputum-smear Microscopy, Sputum-culture And The Xpert Mtb/rif Assay

Sputum-smear microscopy was conducted at the NIDCH or BIRDEM, while Mtb culture was performed in the Mycobacteriology laboratory at icddr,b. Ziehl-Neelsen staining was applied to detect and grade Acid fast bacilli by smear microscopy. Decontaminated sputum pellets were cultured on Lowenstein-Jensen (L-J) slants, which were examined weekly until Mtb colonies were detected to confirm the diagnosis of TB disease. The Xpert MTB/RIF assay was performed using sputum from all TB patients at NIDCH or BIRDEM to rule out rifampicin resistance in the patients following NTP guidelines in Bangladesh. Drug susceptibility testing was not done in the isolated Mtb strains as it is not a requirement by NTP.

Chest X-ray

For lung pathology analysis, a semi-quantitative visual scoring system of 2-dimensional chest x-rays was used as previously reported [31, 32] with some modifications. The lung field was divided into three zones: upper, mid and lower zones. Presence of nodules, patchy or confluent consolidations and cavitations were recorded in each of the three zones. The effusion volume, extent of opacification, cavitation or additional pathology were graded as the percentage of the affected lung. The percent (%) affected area in each zone was scored numerically as: no involvement (0 points); 0.1–33.3% (1 point); 33.4–66.6% (2

points); 66.7–100% (3 points) of pulmonary involvement; finally, the total percentage of the affected lung was calculated.

Mrna Extraction And Real-time Pcr

mRNA analysis of PBMCs and sputum cells was conducted to assess inflammation in the peripheral circulation compared to the local site of Mtb infection. mRNA was extracted using Ambion RiboPure RNA extraction kit (Life Technologies, Vilnius, Lithuania). cDNA conversion was performed using a SuperScript cDNA conversion kit (Invitrogen, Carlsbad, CA). The target genes (CD4, CD8, IL-10, IL-1β, TNF-α and matrix metalloproteinase (MMP)-9, and house-keeping gene (18srRNA) were amplified using Taqman gene expression mastermix and primers in the QuantStudio 5 Real-Time PCR System (Applied Biosystems, Foster City, CA). The cycle threshold (Ct) values for target genes were normalized to 18srRNA and relative mRNA expression was determined using the $2^{-\Delta\Delta CT}$ method. mRNA analysis of sputum samples was performed at baseline, month-1 and month- 2, since most TB patients were unable to produce sputum after 6 months.

Sample Size Calculation

This was an exploratory pilot study, where we aimed to investigate immunological responses in patients with TB-DM compared to TB patients. In general, simulation studies have identified samples size requirements of > 20 for conducting more power-full parametric analyses even when the data is non-normally distributed. Human clinical data tends to be variable, involving patients or volunteers > 30. Based on our previous experiences of TB studies in high-endemic countries [33–37], inclusion of patient numbers > 20 in each sub-group enabled parametric analyses, while smaller sub-group analyses were performed using non-parametric methods. Assuming an initial sample size of 30, accounting for loss to follow-up of 15% and low quality or insufficient clinical materials in another 10% of the patients, we used a sample size of n = 40 in the TB-DM and TB groups.

Statistical analysis

Data is presented as mean and standard deviation (SD) or median and interquartile range (IQR) for continuous, and numbers with percentages for categorical variables. Statistical significance was determined using non-parametric Kruskal-Wallis test with Dunn's post-test (comparing more than two unmatched groups), Mann-Whitney test (comparing two unmatched groups) and Friedman test with Dunn's multiple comparison test (comparing non-parametric one-way repeated measurements). The Chi-square test was used to compare the proportion of patients having TB symptoms between TB and TB-DM groups at baseline. Multivariable regression model and GEE (related to lung pathology only) were used to estimate the difference in outcome variables between the TB-DM and TB groups, and data was adjusted for covariates (age, sex, BCG vaccination status, baseline body mass index (BMI) and SES score). The GEE model (beta (β) value and 95% confidence interval (CI)) were created with an interaction (follow-up

month × groups) to analyze changes in blood hemagram markers and percent lung involvement across time by group status. This analysis was performed on baseline, and three follow up visits, with the following predictors: Time (months- 0, 1, 2 and 6), group, and an interaction term of these two (visit month × group). Covariates that influenced the model R^2 by 5% or more, were selected to avoid colinearity. To evaluate within group changes in TB score, BMI, radiological feature, blood glucose, HbA1c and CBC, the analyses were performed by using 2-way repeated measure Analysis of variance (ANOVA). Spearman ´s correlation test was used for the correlation analyses. Stata/IC (v.13, Stata Corp., LP, College Station, Texas, USA) and GraphPad Prism 7.05 were used for statistical analysis. A *P*-value < 0.05 was considered as significant.

Results

Baseline characteristics

Enrollment of TB patients with or without DM is illustrated in Fig. 1; the demographic, clinical and hematological profiles are shown in Table 1, tables S1 and S2. Forty patients were enrolled in each group; 35 TB and 36 TB-DM patients were followed up to 6 months of anti-TB treatment (Fig. 1). None of the patients were found to be resistant to rifampicin by GeneXpert MTB/RIF and all received standard anti-TB therapy according to the national TB programme (NTP) guidelines. TB-DM patients were significantly older with better SES compared to TB patients. BMI was significantly higher in TB-DM compared to TB group, although both groups had lower BMI compared to the healthy controls. Blood hemogram at enrolment demonstrated that ESR, total white blood cell (WBC) and neutrophil counts were significantly elevated, whereas lymphocyte counts were significantly reduced in both TB and TB-DM patients compared to healthy controls (Table S2). Differences in TB compared to TB-DM patients included significantly higher hemoglobin (Hb) in the TB-DM group, whereas monocyte count was higher in the TB group.

Poor Glycemic Control In Tb-dm Patients

TB-DM patients had significantly higher levels of fasting blood glucose and HbA1c at enrollment compared to TB patients and healthy controls (Fig. 2A-B). Blood glucose levels were further elevated in TB-DM patients after initiation of anti-TB chemotherapy compared to baseline (Fig. 2A). Likewise, HbA1c remained elevated in TB-DM patients at the follow up visits (Fig. 2B). Insulin levels increased gradually in TB-DM patients after start of anti-TB treatment and were significantly higher compared to TB patients at all visits (Fig. 2C). However, insulin and C-peptide levels were relatively lower in both patient groups compared to healthy controls (Fig. 2C-D). These results suggested that TB-DM patients maintained poor glycemic control at TB diagnosis and throughout anti-TB treatment.

Similar changes in TB symptoms and sputum culture conversion in TB and TB-DM patients upon anti-TB treatment

Treatment outcome was similar in both groups of patients; 90% of the TB-DM patients and 87.5% of the TB patients were cured after 6 months of anti-TB treatment. All patients experienced typical TB symptoms such as fever and cough (Table S1). TB patients more often had chest pain, loss of appetite, nausea, anemia and low BMI, while TB-DM patients experienced night sweats more often. BMI steadily increased in both patient groups upon anti-TB treatment but remained significantly higher in TB-DM patients compared with TB patients (Fig. 3A). Both groups had a significant reduction of the composite TB score after start of anti-TB chemotherapy (Fig. 3B).

Majority of the TB patients had positive Mtb sputum smear (Table 1) and culture results. After 1 month of anti-TB treatment, the proportion of TB-DM patients with negative Mtb culture results were similar to TB patients (26/36 = 72% versus 20/35 = 57%, respectively, $P = 0.11$ by chi-square test). After 2 months, 97% and 95% of TB-DM and TB patients, respectively had a negative Mtb sputum culture ($P = 0.98$), while all patients were Mtb culture negative after 6 months of treatment.

Slower Radiological Improvement In Tb-dm Patients After Anti-tb Treatment

Assessment of pulmonary pathology using chest X-ray and quantification of inflammatory lesions in the different zones of the left and right lungs, revealed that anti-TB treatment gradually reduced pulmonary inflammation in both TB and TB-DM patients (Fig. 4). Total lung involvement was significantly higher in TB-DM compared to TB patients at baseline, but not at follow up as assessed by Mann-Whitney U-tests (Fig. 4A). No difference in lung involvement was detected in the upper zone (Fig. 4B). However, lung involvement in the middle zones of the lungs in the TB-DM patients was significantly higher at baseline and at months-1 and − 2 (Fig. 4C) and in the lower zones at month-6 (Fig. 4D) compared to the TB group. Multivariate regression analysis showed significantly higher level of % lung involvement in the whole lung, as well as in the middle and lower zones in TB-DM patients compared to TB patients at baseline (Table S3). Significantly elevated pulmonary involvement in the middle and lower zones, and in the whole lung was also evident in the longitudinal analysis by GEE model (Table S3).

Lung pathology is associated with hemogram parameters and HbA1c levels

Upon anti-TB treatment, both patient groups demonstrated decreased ESR, WBC, neutrophil, monocytes and platelet counts as well as NLR, monocyte-to lymphocyte (MLR) and PLR, but increased hemoglobin

levels and lymphocyte counts (Table S2). Multiple regression analysis showed significantly higher WBC count at month-6 in TB-DM group compared to TB patients (Table S2).

Lung pathology in TB-DM patients was inversely associated with hemoglobin levels and lymphocyte counts, and positively associated with ESR, WBC, neutrophil count, platelet count and PLR (Table 2). In TB patients, there was an inverse association between pathological lung involvement and hemoglobin levels and a positive association with WBC count, platelet count and PLR (Table 2). Patient groups combined showed a strong association between HbA1c and % lung involvement (β = 1.01, 95% CI=-0.19, 2.22; *P* = 0.09).

Table 2
Longitudinal association of lung involvement with TB score and blood hemogram markers in study patients

	% lung involvement			
	TB (n = 35)		TB-DM (n = 36)	
	β-coefficient (95% CI)	p-value	β-coefficient (95% CI)	p-value
TB score	0.20(-0.02, 0.42)	0.073	0.13(-0.14, 0.39)	0.362
ESR	0.03(-0.0003, 0.05)	0.053	0.06(0.03, 0.09)	< 0.001
Hb	-0.34(-0.66, -0.01)	0.041	-1.15(-1.80, -0.49)	0.001
WBC	0.32(0.08, 0.56)	0.008	0.41(0.13, 0.69)	0.004
Lymphocyte	-0.05(-01.2, 0.02)	0.173	-0.14(-0.23, -0.05)	0.002
Neutrophils	0.06(-0.003, 0.13)	0.060	0.11(0.04, 0.18)	0.002
Monocyte	-0.22(-0.64, 0.20)	0.298	0.01(-0.40, 0.41)	0.980
Platelet	0.02(0.01, 0.04)	0.001	0.02(0.01, 0.03)	0.005
NLR	0.09(-0.02, 0.21)	0.121	0.10(-0.11, 0.31)	0.353
MLR	8.53(-2.43, 19.49)	0.127	6.07(-0.78, 12.93)	0.082
PLR	0.17(0.07, 0.27)	0.001	0.12(0.04, 0.20)	0.003

Data were analyzed using Generalized estimating equation (GEE), and the results are expressed as beta β-coefficient and 95% confidence interval (CI). The GEE model was adjusted by age, sex, baseline BMI, SES score, BCG vaccination status and time (to reduce multicollinearity).The percent affected area in each zone of the lung was scored numerically as: no involvement (0 points); 0.1–33.3% (1 point); 33.4–66.6% (2 points); 66.7–100% (3 points) of pulmonary involvement; finally, the total percentage of the affected lung was calculated. BCG: *Bacillus Calmette–Guérin;* BMI: body mass index; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; SES: socioeconomic status; WBC: white blood cell.

mRNA expression profiling suggests higher level of inflammation in TB-DM patients after anti-TB treatment

Analysis by Mann-Whitney U-test did not reveal any significant differences in the expression of CD4 and CD8 transcripts in PBMC in TB-DM compared to TB patients (Fig. 5A-B). In sputum cells, CD4 mRNA was significantly lower in TB-DM compared to TB patients both at baseline and after 1 month of anti-TB treatment (Fig. 5F), while the expression of CD8 transcripts was significantly higher in TB-DM patients at month-2 (Fig. 5G). Expression of the pro-inflammatory cytokines IL-1β and TNF-α in PBMCs decreased in the TB patients after 1 month of anti-TB treatment, resulting in a significantly lower expression in the TB compared to the TB-DM group (Fig. 5C-D). For IL-1β, this difference remained after 2 months of anti-TB treatment (Fig. 5C). Contrary to that, there were no differences in IL-1β or TNF-α transcripts between the groups in sputum at any time point (Fig. 5H-I). The tissue degrading enzyme MMP9, was relatively higher in both PBMC and sputum cells from TB-DM patients at baseline and at follow up compared to TB patients, although this trend was not significant (data not shown). Peripheral expression of the anti-inflammatory cytokine IL-10 was similar in TB-DM and TB patients at baseline, but TB-DM patients exhibited a significantly higher peripheral expression of IL-10 at month-2 compared to TB patients (Fig. 5E). In contrary, a significantly lower IL-10 expression in sputum cells was observed in TB-DM patients at month-1 and − 2 (Fig. 5J). Regression analyses confirmed that sputum CD8 at month-2 and TNF-α transcripts at month-1 were elevated, while IL-10 were lower at month-2 in TB-DM compared to TB patients (Table 3). In addition, peripheral IL-10 transcripts at month-1 and − 2 were significantly higher in TB-DM patients (Table 3). Furthermore, correlation analyses showed that high blood glucose and HbA1c levels were inversely correlated to IL-10 transcripts in sputum cells from both patient groups after 1 and 2 months of anti-TB treatment (Fig. 6A-F). These results suggest that TB-induced inflammation was not as effectively reduced in TB-DM as compared to TB patients after start of anti-TB treatment, but low-grade inflammation persisted in TB-DM patients.

Discussion

This longitudinal study explored the clinical, radiological and immune responses in patients with TB-DM compared to TB disease before and after start of anti-TB treatment. TB-DM patients displayed a greater degree of lung pathology in the middle and lower lung zones compared to TB alone after onset of TB chemotherapy, despite similar clinical and microbiological responses to anti-TB treatment. A slower radiological improvement in TB-DM patients was accompanied with elevated pro-inflammatory IL-1b and TNF-a as well as anti-inflammatory IL-10 transcripts in PBMC, while a higher expression of CD8 and TNF-α but lower CD4 and IL-10 transcripts was observed in the lung. These results suggest that non-resolving systemic and local inflammation likely represent persistent low-grade inflammation and enhanced pulmonary involvement in TB-DM comorbidity that may contribute to lung damage resulting from impaired counter regulatory mechanisms.

It was evident that TB-DM patients experienced poor glycemic control throughout the study period. Previous studies have found that TB infection can contribute to impaired glucose tolerance and worsen glycemic control in diabetic patients, and even cause new onset of diabetes [42, 43]. Elevated levels of fasting blood glucose and HbA1c in the present study could also be due to poor compliance with diabetic drugs and/or exercise and dietary advice.

We did not observe any difference in sputum-smear positivity at enrollment, although a higher bacterial burden in sputum from TB-DM patients at diagnosis has frequently been observed [7, 10, 44]. Previously, it has been described that TB-DM co-morbidity could result in higher susceptibility to TB [1, 42], more severe clinical symptoms, and slow response to anti-TB treatment including delayed sputum-culture conversion [6, 7, 44-46]. However, a few other studies reported no difference in sputum conversion rates or final treatment outcome between TB and TB-DM patients after anti-TB therapy [9, 10]. In the present study, sputum or culture conversion was not delayed in TB-DM patients compared to TB patients, and accordingly treatment outcome did not differ between the groups. Severity of clinical symptoms of TB at baseline and during anti-TB treatment was similar in TB and TB-DM patients, and both groups demonstrated significant disease remission after start of treatment as reported in other studies [5, 47, 48]. Consistent with other reports, TB-DM patients more often had a higher socioeconomic status [49], and comorbidity was associated with higher age and BMI compared to TB alone [5, 50, 51]. However, the potential age-related immune dysfunctions or other physiological variables, generaly associated with older age groups (e.g. >65 years) are less likely to occur in the present cohort, where the median age range is from 26.6 years to 40.1 years [52]. Although, our data analyses were adjusted for these confounders, the differences in baseline demographics may still have influenced the treatment outcome in this patient cohort. It was recently observed that TB patients in India with a low BMI had considerably longer time to sputum culture conversion, and enhanced rates of treatment failure and death as compared to patients with a normal-to-high BMI, independently of DM status [53]. Thus, demographic variables such as BMI or SES might obscure the negative effects of DM on TB.

It has been demonstrated that TB-DM patients exhibit atypical radiographic findings including high frequency of lower lung involvement and more advanced forms of cavitary disease [54-56]. This study also observed an enhanced magnitude and duration of pathological lesions in the lung, especially in the middle and lower zones of the TB-DM patients (Fig. 4 and Table S3). However, neither TB-DM nor TB patients had cavitary TB, which is a deviation from the radiological findings in other studies on TB-DM disease. Absence of lung cavities could support less advanced TB disease in this cohort, which may also account for the lack of differences in the clinical and bacteriological response in TB-DM compared to TB patients. Some studies suggest that uncontrolled DM (HbA1c>7%) is associated with TB disease progression including enhanced pathological lung involvement, despite no differences in clinical symptoms or sputum smear results between diabetic and non-diabetic TB patients [48, 54, 57]. We found

a significant positive association of HbA1c levels and lung pathology when both TB and TB-DM patient groups were combined. Our observations suggest that the TB-DM patients enrolled in this study with either controlled or persistent hyperglycemia of ≤5 years' duration without other associated complications, had manifestations of a relatively mild TB disease, which could reduce the possibilities to detect clinically relevant differences in TB symptoms and bacteriological outcomes compared with non-diabetic TB patients. In spite of this, the extent of lung pathology and local immune responses in the respiratory tract may be different in TB-DM compared to TB patients, which could represent early immune deviations of clinical TB disease in TB-DM patients. According to the guidelines of the Diabetic Association of Bangladesh (DAB), all DM patients attending BIRDEM or DAB linked centers/clinics are routinely asked about respiratory symptoms and accordingly screened for pulmonary TB and hence are diagnosed for TB in the early phase of disease. Thus, an early diagnosis and rapid implementation of adequate anti-TB treatment is likely important for the outcome of TB-DM disease.

According to the mRNA data (Fig. 5), peripheral expression of proinflammatory cytokines TNF-a and IL-1b, as well as anti-inflammatory IL-10 was higher in TB-DM compared to TB patients after anti-TB treatment. These differences are primarily due to down-regulated levels of these cytokines in TB patients but sustained expression in TB-DM disease. Excess IL-1b has been shown to promote neutrophil accumulation in the lung of active TB patients, which may exacerbate lung damage and disease progression [15]. Interestingly, high-fat diet induces an inflammatory IL-1b response that impairs insulin signaling and results in reduced glucose tolerance and insulin sensitivity, which is a hallmark of type 2 DM [58]. Accordingly, pharmacological blockade of IL-1b in patients with type 2 DM improved beta-cell secretory function and reduced hyperglycemia and markers of systemic inflammation [59]. Lower levels of CD4 and IL-10 but higher CD8 and TNF-α mRNA in the lungs of TB-DM patients after treatment are other important findings of the study, which could probably explain persistence of pulmonary inflammation during anti-TB therapy. It is likely that TB-DM patients may not down-regulate pro-inflammatory responses as effectively because of insufficient IL-10 production locally in the lungs. Enhanced IL-10 levels in the periphery but reduced IL-10 in sputum cells from TB-DM patients at follow up, may also indicate that IL-10 producing cells fail to properly migrate from the peripheral circulation to the Mtb-infected lung. Low IL-10 production capacity of immune cells and reduced anti-inflammatory function of IL-10 have been reported in TB-DM disease compared to TB alone [60, 61]. While IL-10 could contribute to unwanted immunosuppression of Mtb-specific immune responses [62, 63], it is also necessary to control local pathological inflammation and sustain an environment that limits Mtb replication [64]. Moreover, hypo-responsiveness to IL-10 signaling has also been shown to contribute to chronic low-grade inflammation in type 2 DM [65]. Similar to our findings, a recent study reported prolonged inflammation and delayed resolution of inflammation due to insufficient expression of anti-inflammatory cytokines including IL-10 in TB-DM compared to TB patients [13]. However, that study demonstrated hyperinflammation and low IL-10 levels already at baseline with a slow resolution of inflammation in TB-DM patients [13]. In influenza virus infection, IL-10 producing CD4 cells have a critical function to limit pulmonary inflammation and tissue injury and therefore, blockade of T-cell derived IL-10

enhanced lethal inflammation without impacting virus titers *in vivo* [66]. Similarly, a decrease of IL-10 producing CD4 cells in the lungs of TB-DM patients may enhance inflammation and disease severity without affecting mycobacterial load in sputum. A recent longitudinal study using positron emission tomography and computerized tomography (PET-CT) to evaluate local lung inflammation in pulmonary TB patients demonstrated enhancement and/or development of new inflammatory lesions in many patients despite 6 months completion of anti-TB therapy and 1 year of follow-up [67]. Patients apparently culture negative at the end of treatment, were found to have Mtb transcripts in sputum and bronchoalveolar lavage, suggesting that persistent bacterial transcription or stabilized mRNA from dormant/dead bacteria may fuel inflammatory reactions in the lung. It is possible that, persistence of lung pathology in TB-DM comorbidity in the present study may result from either non-resolving sterile inflammation (high TNF-α and low IL-10 in the lungs) or persistence of non-replicating Mtb due to inefficient efferocytosis leading to inflammation [68, 69]. Further studies should confirm whether low IL-10 levels but enhanced proinflammatory cytokines could fuel lung pathology in TB-DM patients and promote the progression towards severe cavitary disease compared to TB patients.

The strengths of this study included longitudinal sampling of both peripheral blood and lung-derived sputum; the limitations were small patient numbers, age and sex imbalances as well as differences in BMI, SES and BCG vaccination status between the groups. According to the National TB prevalence survey 2015-2016, the male to female ratio of bacteriologically confirmed TB cases in Bangladesh are 2-3 time higher in males, which could explain a high enrollment of males in this study. Furthermore, the inclusion/exclusion criteria (e.g. estimated duration of DM was limited to 5 years) used in this study hampered complete matching of demographic parameters and therefore patient enrollment ended up somewhat biased, which also could have affected the outcome. However, TB-DM patients with hyperglycemia of ≤5 years' duration allowed us to detect early inflammatory changes in TB-DM cases that may contribute to severe effects such as cavitary disease in the long term when left untreated. In addition, follow-up of TB-DM patients at 6 months instead of 9 months anti-TB therapy, which could have increased the likelihood to detect clinically significant differences between the groups. Another limitation is collection of spontaneous sputum samples, as expectorated sputum, especially at the later time point (2 month) may contain cells mostly from the upper airways including both immune cells and oropharyngeal epithelial cells. Additionally, mRNA expression of phenotype markers and cytokines in the cells may not correlate directly to protein expression of the same markers. Although, we have previously found mRNA transcripts to represent a good complementary measure to protein expression in tissue samples obtained from TB infected patients [37, 41].

Conclusion

TB-DM disease was associated with a greater degree of pathological lung involvement in the lower lung areas after standard anti-TB treatment, despite similar clinical TB symptoms and sputum conversion rates. The results suggest that DM may reduce control of persistent inflammation in TB by insufficient IL-

10 production locally in the lung, which could result in pulmonary impairment and aggravate TB-DM disease.

List Of Abbreviations

AFB: Acid fast bacilli; BMI: Body mass index; CBC: Complete Blood Count; DM: Diabetes mellitus; DOTS: Directly observed therapy short-course; ESR: Erythrocyte sedimentation rate; GEE: Generalized estimating equation; GIP: Gastric inhibitory polypeptide; GLP: Glucagon-Like Peptide; HbA1c: Hemoglobin A1c; MLR: monocyte-to-lymphocyte ratio; MMP: Matrix metalloproteinase; Mtb: *Mycobacterium tuberculosis;* NLR: Neutrophil-to-lymphocyte ratio; PAI-1: Plasminogen activator inhibitor 1; PBMC: Peripheral blood mononuclear cells; PLR: platelet-to-lymphocyte ratio; SES: Socio-economic status; IL: interleukin; TB: Tuberculosis; TNF: Tumor necrosis factor; WBC: White Blood Cells

Declarations

Ethics approval and consent to participate

The study was approved by the Research Review Committee (RRC) and the Ethical Review Committee (ERC) at the icddrb, in Dhaka, Bangladesh (protocol PR-13074) and by the Ethical Review Board (EPN) in Stockholm, Sweden (protocol 2019-01622). Written informed consent was obtained from all patients before enrollment in accordance with the Helsinki Declaration.

Consent for publication

Not applicable

Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Swedish Research Council´s (VR) Project Research Grant in Development Research (2014-02592 to SB), Swedish Heart and Lung Foundation (HLF) (2016-0470 and 2016-0815 to SB), BADAS-USAID TB Care II Project Bangladesh, BADAS-USAID Challenge TB Bangladesh Project (to MDH) and PATH (to RR, salary support of staff) and icddr,b (RR). icddr,b acknowledges with gratitude the commitment of VR, HLF, BADAS, USAID, Global Fund and PATH to its research efforts. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support

Authors' contributions

RR, SB and BA generated the scientific hypothesis. RR, SB, PS and AM prepared the project plan and planned and designed the clinical protocols and ex vivo experiments. MDH, SMMK contributed to patient evaluation, recruitment and clinical sample collection. IT, AM and PS performed the experiments on clinical samples. RR, SB, BA, MAH, AM and PS analyzed the data and wrote the manuscript. MAH and AM prepared figures. All authors read the final manuscript version.

Acknowledgements

We express our sincere thanks to Dr. ASM Arifuzzaman, Dr. Taslima Akhter, Dr Adity Roy, Saiful Islam (icddr,b), Shamim Hossain (NIDCH) and Dr. Md. Afsarul Habib, Dr. Farhana Hoque Khan & Dr. Ferdousi Begum (BIRDEM) for their support in patient enrollment and collecting data.

References

1. Jeon CY, Murray MB: **Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies**. *PLoS Med* 2008, **5**(7):e152.
2. Wild S, Roglic G, Green A, Sicree R, King H: **Global prevalence of diabetes: estimates for the year 2000 and projections for 2030**. *Diabetes care* 2004, **27**(5):1047-1053.
3. Huangfu P, Ugarte-Gil C, Golub J, Pearson F, Critchley J: **The effects of diabetes on tuberculosis treatment outcomes: an updated systematic review and meta-analysis**. *Int J Tuberc Lung D* 2019, **23**(7):783-796.
4. Chiang CY, Bai KJ, Lin HH, Chien ST, Lee JJ, Enarson DA, Lee TI, Yu MC: **The influence of diabetes, glycemic control, and diabetes-related comorbidities on pulmonary tuberculosis**. *PloS one* 2015, **10**(3):e0121698.
5. Dousa KM, Hamad A, Albirair M, Al Soub H, Elzouki AN, Alwakeel MI, Thiel BA, Johnson JL: **Impact of Diabetes Mellitus on the Presentation and Response to Treatment of Adults With Pulmonary Tuberculosis in Qatar**. *Open Forum Infect Dis* 2019, **6**(1):ofy335.
6. Leung CC, Yew WW, Mok TYW, Lau KS, Wong CF, Chau CH, Chan CK, Chang KC, Tam G, Tam CM: **Effects of diabetes mellitus on the clinical presentation and treatment response in tuberculosis**. *Respirology* 2017, **22**(6):1225-1232.
7. Restrepo BI, Fisher-Hoch SP, Crespo JG, Whitney E, Perez A, Smith B, McCormick JB, Nuevo Santander Tuberculosis T: **Type 2 diabetes and tuberculosis in a dynamic bi-national border population**. *Epidemiol Infect* 2007, **135**(3):483-491.
8. Shariff NM, Safian N: **Diabetes mellitus and its influence on sputum smear positivity at the 2nd month of treatment among pulmonary tuberculosis patients in Kuala Lumpur, Malaysia: A case control study**. *International journal of mycobacteriology* 2015, **4**(4):323-329.

9. Prasad P, Gounder S, Varman S, Viney K: **Sputum smear conversion and treatment outcomes for tuberculosis patients with and without diabetes in Fiji**. *Public Health Action* 2014, **4**(3):159-163.
10. Singla R, Khan N, Al-Sharif N, Ai-Sayegh MO, Shaikh MA, Osman MM: **Influence of diabetes on manifestations and treatment outcome of pulmonary TB patients**. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2006, **10**(1):74-79.
11. Kumar NP, Banurekha VV, Nair D, Sridhar R, Kornfeld H, Nutman TB, Babu S: **Coincident pre-diabetes is associated with dysregulated cytokine responses in pulmonary tuberculosis**. *PloS one* 2014, **9**(11):e112108.
12. Restrepo BI, Fisher-Hoch SP, Pino PA, Salinas A, Rahbar MH, Mora F, Cortes-Penfield N, McCormick JB: **Tuberculosis in poorly controlled type 2 diabetes: altered cytokine expression in peripheral white blood cells**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2008, **47**(5):634-641.
13. Kumar NP, Fukutani KF, Shruthi BS, Alves T, Silveira-Mattos PS, Rocha MS, West K, Natarajan M, Viswanathan V, Babu S *et al*: **Persistent inflammation during anti-tuberculosis treatment with diabetes comorbidity**. *Elife* 2019, **8**.
14. Ferlita S, Yegiazaryan A, Noori N, Lal G, Nguyen T, To K, Venketaraman V: **Type 2 Diabetes Mellitus and Altered Immune System Leading to Susceptibility to Pathogens, Especially Mycobacterium tuberculosis**. *Journal of clinical medicine* 2019, **8**(12).
15. Zhang G, Zhou B, Li S, Yue J, Yang H, Wen Y, Zhan S, Wang W, Liao M, Zhang M *et al*: **Allele-specific induction of IL-1beta expression by C/EBPbeta and PU.1 contributes to increased tuberculosis susceptibility**. *PLoS pathogens* 2014, **10**(10):e1004426.
16. Couper KN, Blount DG, Riley EM: **IL-10: the master regulator of immunity to infection**. *Journal of immunology* 2008, **180**(9):5771-5777.
17. Beamer GL, Flaherty DK, Assogba BD, Stromberg P, Gonzalez-Juarrero M, de Waal Malefyt R, Vesosky B, Turner J: **Interleukin-10 promotes Mycobacterium tuberculosis disease progression in CBA/J mice**. *Journal of immunology* 2008, **181**(8):5545-5550.
18. Redford PS, Murray PJ, O'Garra A: **The role of IL-10 in immune regulation during M. tuberculosis infection**. *Mucosal immunology* 2011, **4**(3):261-270.
19. Martens GW, Arikan MC, Lee J, Ren F, Greiner D, Kornfeld H: **Tuberculosis susceptibility of diabetic mice**. *Am J Respir Cell Mol Biol* 2007, **37**(5):518-524.
20. Vallerskog T, Martens GW, Kornfeld H: **Diabetic mice display a delayed adaptive immune response to Mycobacterium tuberculosis**. *Journal of immunology* 2010, **184**(11):6275-6282.
21. Kumar NP, Moideen K, George PJ, Dolla C, Kumaran P, Babu S: **Coincident diabetes mellitus modulates Th1-, Th2-, and Th17-cell responses in latent tuberculosis in an IL-10- and TGF-beta-dependent manner**. *Eur J Immunol* 2016, **46**(2):390-399.
22. Yamashiro S, Kawakami K, Uezu K, Kinjo T, Miyagi K, Nakamura K, Saito A: **Lower expression of Th1-related cytokines and inducible nitric oxide synthase in mice with streptozotocin-induced diabetes**

mellitus infected with Mycobacterium tuberculosis. *Clin Exp Immunol* 2005, **139**(1):57-64.

23. Al-Attiyah RJ, Mustafa AS: **Mycobacterial antigen-induced T helper type 1 (Th1) and Th2 reactivity of peripheral blood mononuclear cells from diabetic and non-diabetic tuberculosis patients and Mycobacterium bovis bacilli Calmette-Guerin (BCG)-vaccinated healthy subjects**. *Clin Exp Immunol* 2009, **158**(1):64-73.
24. Kumar NP, Sridhar R, Nair D, Banurekha VV, Nutman TB, Babu S: **Type 2 diabetes mellitus is associated with altered CD8(+) T and natural killer cell function in pulmonary tuberculosis**. *Immunology* 2015, **144**(4):677-686.
25. Wang X, Ma A, Han X, Chan L, Liang H, Litifu A, Xue F: **T Cell Profile was Altered in Pulmonary Tuberculosis Patients with Type 2 Diabetes**. *Med Sci Monit* 2018, **24**:636-642.
26. Kumar NP, Moideen K, Viswanathan V, Kornfeld H, Babu S: **Effect of standard tuberculosis treatment on naive, memory and regulatory T-cell homeostasis in tuberculosis-diabetes co-morbidity**. *Immunology* 2016, **149**(1):87-97.
27. Lachmandas E, Vrieling F, Wilson LG, Joosten SA, Netea MG, Ottenhoff TH, van Crevel R: **The effect of hyperglycaemia on in vitro cytokine production and macrophage infection with Mycobacterium tuberculosis**. *PloS one* 2015, **10**(2):e0117941.
28. Raposo-Garcia S, Guerra-Laso JM, Garcia-Garcia S, Juan-Garcia J, Lopez-Fidalgo E, Diez-Tascon C, Nebreda-Mayoral T, Lopez-Medrano R, Rivero-Lezcano OM: **Immunological response to Mycobacterium tuberculosis infection in blood from type 2 diabetes patients**. *Immunol Lett* 2017, **186**:41-45.
29. Beckman JA, Creager MA: **Vascular Complications of Diabetes**. *Circ Res* 2016, **118**(11):1771-1785.
30. Saha KK, Frongillo EA, Alam DS, Arifeen SE, Persson LA, Rasmussen KM: **Appropriate infant feeding practices result in better growth of infants and young children in rural Bangladesh**. *Am J Clin Nutr* 2008, **87**(6):1852-1859.
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: **Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.** *Diabetologia* 1985, **28**(7):412-419.
32. Ohkura T, Shiochi H, Fujioka Y, Sumi K, Yamamoto N, Matsuzawa K, Izawa S, Kinoshita H, Ohkura H, Kato M *et al*: **20/(fasting C-peptide × fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report.** *Cardiovasc Diabetol* 2013, **12**(21).
33. Rudolf F: **The Bandim TBscore - reliability, further development, and evaluation of potential uses**. *Global Health Action* 2014, **7**:1-13.
34. Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, Sodemann M: **TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course**. *Scand J Infect Dis* 2008, **40**(2):111-120.
35. Kurashima AM, M.; Horibe, M,; Hoshino, Y.; Shiraishi, Y.; Kudoh, S.: **A Method for Visual Scoring of Pulmonary Mycobacterium Avium Complex Disease: "NICE Scoring System"**. *Mycobacterial Diseases*

2013, **3**(1).

36. Ralph AP, Ardian M, Wiguna A, Maguire GP, Becker NG, Drogumuller G, Wilks MJ, Waramori G, Tjitra E, Sandjaja *et al*: **A simple, valid, numerical score for grading chest x-ray severity in adult smear-positive pulmonary tuberculosis**. *Thorax* 2010, **65**(10):863-869.
37. Andersson J, Samarina A, Fink J, Rahman S, Grundstrom S: **Impaired expression of perforin and granulysin in CD8+ T cells at the site of infection in human chronic pulmonary tuberculosis**. *Infect Immun* 2007, **75**(11):5210-5222.
38. Ashenafi S, Aderaye G, Bekele A, Zewdie M, Aseffa G, Hoang AT, Carow B, Habtamu M, Wijkander M, Rottenberg M *et al*: **Progression of clinical tuberculosis is associated with a Th2 immune response signature in combination with elevated levels of SOCS3**. *Clin Immunol* 2014, **151**(2):84-99.
39. Ashenafi S, Aderaye G, Zewdie M, Raqib R, Bekele A, Magalhaes I, Lema B, Habtamu M, Rekha RS, Aseffa G *et al*: **BCG-specific IgG-secreting peripheral plasmablasts as a potential biomarker of active tuberculosis in HIV negative and HIV positive patients**. *Thorax* 2013, **68**(3):269-276.
40. Rahman S, Gudetta B, Fink J, Granath A, Ashenafi S, Aseffa A, Derbew M, Svensson M, Andersson J, Brighenti SG: **Compartmentalization of immune responses in human tuberculosis: few CD8+ effector T cells but elevated levels of FoxP3+ regulatory t cells in the granulomatous lesions**. *Am J Pathol* 2009, **174**(6):2211-2224.
41. Rahman S, Rehn A, Rahman J, Andersson J, Svensson M, Brighenti S: **Pulmonary tuberculosis patients with a vitamin D deficiency demonstrate low local expression of the antimicrobial peptide LL-37 but enhanced FoxP3+ regulatory T cells and IgG-secreting cells**. *Clin Immunol* 2015, **156**(2):85-97.
42. Dooley KE, Chaisson RE: **Tuberculosis and diabetes mellitus: convergence of two epidemics**. *Lancet Infect Dis* 2009, **9**(12):737-746.
43. Yorke E, Atiase Y, Akpalu J, Sarfo-Kantanka O, Boima V, Dey ID: **The Bidirectional Relationship between Tuberculosis and Diabetes**. *Tuberculosis research and treatment* 2017, **2017**:1702578.
44. Chang JT, Dou HY, Yen CL, Wu YH, Huang RM, Lin HJ, Su IJ, Shieh CC: **Effect of type 2 diabetes mellitus on the clinical severity and treatment outcome in patients with pulmonary tuberculosis: a potential role in the emergence of multidrug-resistance**. *J Formos Med Assoc* 2011, **110**(6):372-381.
45. Dooley KE, Tang T, Golub JE, Dorman SE, Cronin W: **Impact of diabetes mellitus on treatment outcomes of patients with active tuberculosis**. *Am J Trop Med Hyg* 2009, **80**(4):634-639.
46. Gil-Santana L, Almeida-Junior JL, Oliveira CA, Hickson LS, Daltro C, Castro S, Kornfeld H, Netto EM, Andrade BB: **Diabetes Is Associated with Worse Clinical Presentation in Tuberculosis Patients from Brazil: A Retrospective Cohort Study**. *PloS one* 2016, **11**(1):e0146876.
47. Workneh MH, Bjune GA, Yimer SA: **Diabetes mellitus is associated with increased mortality during tuberculosis treatment: a prospective cohort study among tuberculosis patients in South-Eastern Amahra Region, Ethiopia**. *Infect Dis Poverty* 2016, **5**:22.
48. Park SW, Shin JW, Kim JY, Park IW, Choi BW, Choi JC, Kim YS: **The effect of diabetic control status on the clinical features of pulmonary tuberculosis**. *Eur J Clin Microbiol Infect Dis* 2012, **31**(7):1305-

1310.

49. Jimenez-Corona ME, Cruz-Hervert LP, Garcia-Garcia L, Ferreyra-Reyes L, Delgado-Sanchez G, Bobadilla-Del-Valle M, Canizales-Quintero S, Ferreira-Guerrero E, Baez-Saldana R, Tellez-Vazquez N *et al*: **Association of diabetes and tuberculosis: impact on treatment and post-treatment outcomes**. *Thorax* 2013, **68**(3):214-220.
50. Alisjahbana B, Sahiratmadja E, Nelwan EJ, Purwa AM, Ahmad Y, Ottenhoff TH, Nelwan RH, Parwati I, van der Meer JW, van Crevel R: **The effect of type 2 diabetes mellitus on the presentation and treatment response of pulmonary tuberculosis**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007, **45**(4):428-435.
51. Ruslami R, Aarnoutse RE, Alisjahbana B, van der Ven AJ, van Crevel R: **Implications of the global increase of diabetes for tuberculosis control and patient care**. *Trop Med Int Health* 2010, **15**(11):1289-1299.
52. Valiathan R, Ashman M, Asthana D: **Effects of Ageing on the Immune System: Infants to Elderly**. *Scand J Immunol* 2016, **83**(4):255-266.
53. Kornfeld H, Sahukar SB, Procter-Gray E, Kumar NP, West K, Kane K, Natarajan M, Li W, Babu S, Viswanathan V: **Impact of Diabetes and Low Body Mass Index on Tuberculosis Treatment Outcomes**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.
54. Huang LK, Wang HH, Lai YC, Chang SC: **The impact of glycemic status on radiological manifestations of pulmonary tuberculosis in diabetic patients**. *PloS one* 2017, **12**(6):e0179750.
55. Magee MJ, Kempker RR, Kipiani M, Gandhi NR, Darchia L, Tukvadze N, Howards PP, Narayan KM, Blumberg HM: **Diabetes mellitus is associated with cavities, smear grade, and multidrug-resistant tuberculosis in Georgia**. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2015, **19**(6):685-692.
56. Umut S, Tosun GA, Yildirim N: **Radiographic location of pulmonary tuberculosis in diabetic patients**. *Chest* 1994, **106**(1):326.
57. Chiang CY, Lee JJ, Chien ST, Enarson DA, Chang YC, Chen YT, Hu TY, Lin CB, Suk CW, Tao JM *et al*: **Glycemic control and radiographic manifestations of tuberculosis in diabetic patients**. *PloS one* 2014, **9**(4):e93397.
58. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP: **Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling**. *Nat Immunol* 2011, **12**(5):408-415.
59. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY: **Interleukin-1-receptor antagonist in type 2 diabetes mellitus**. *N Engl J Med* 2007, **356**(15):1517-1526.
60. Lopez-Lopez N, Martinez AGR, Garcia-Hernandez MH, Hernandez-Pando R, Castaneda-Delgado JE, Lugo-Villarino G, Cougoule C, Neyrolles O, Rivas-Santiago B, Valtierra-Alvarado MA *et al*: **Type-2 diabetes alters the basal phenotype of human macrophages and diminishes their capacity to respond, internalise, and control Mycobacterium tuberculosis**. *Mem Inst Oswaldo Cruz* 2018, **113**(4):e170326.

61. Tsukaguchi K, Okamura H, Ikuno M, Kobayashi A, Fukuoka A, Takenaka H, Yamamoto C, Tokuyama T, Okamoto Y, Fu A *et al*: **[The relation between diabetes mellitus and IFN-gamma, IL-12 and IL-10 productions by CD4+ alpha beta T cells and monocytes in patients with pulmonary tuberculosis]**. *Kekkaku* 1997, **72**(11):617-622.
62. Almeida AS, Lago PM, Boechat N, Huard RC, Lazzarini LC, Santos AR, Nociari M, Zhu H, Perez-Sweeney BM, Bang H *et al*: **Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity**. *Journal of immunology* 2009, **183**(1):718-731.
63. Moreira-Teixeira L, Redford PS, Stavropoulos E, Ghilardi N, Maynard CL, Weaver CT, Freitas do Rosario AP, Wu X, Langhorne J, O'Garra A: **T Cell-Derived IL-10 Impairs Host Resistance to Mycobacterium tuberculosis Infection**. *Journal of immunology* 2017, **199**(2):613-623.
64. Higgins DM, Sanchez-Campillo J, Rosas-Taraco AG, Lee EJ, Orme IM, Gonzalez-Juarrero M: **Lack of IL-10 alters inflammatory and immune responses during pulmonary Mycobacterium tuberculosis infection**. *Tuberculosis* 2009, **89**(2):149-157.
65. Barry JC, Shakibakho S, Durrer C, Simtchouk S, Jawanda KK, Cheung ST, Mui AL, Little JP: **Hyporesponsiveness to the anti-inflammatory action of interleukin-10 in type 2 diabetes**. *Sci Rep* 2016, **6**:21244.
66. Sun J, Madan R, Karp CL, Braciale TJ: **Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10**. *Nature medicine* 2009, **15**(3):277-284.
67. Malherbe ST, Shenai S, Ronacher K, Loxton AG, Dolganov G, Kriel M, Van T, Chen RY, Warwick J, Via LE *et al*: **Persisting positron emission tomography lesion activity and Mycobacterium tuberculosis mRNA after tuberculosis cure**. *Nature medicine* 2016, **22**(10):1094-1100.
68. Gan YH: **Host susceptibility factors to bacterial infections in type 2 diabetes**. *PLoS pathogens* 2013, **9**(12):e1003794.
69. Lecube A, Pachon G, Petriz J, Hernandez C, Simo R: **Phagocytic activity is impaired in type 2 diabetes mellitus and increases after metabolic improvement**. *PloS one* 2011, **6**(8):e23366.

Figures

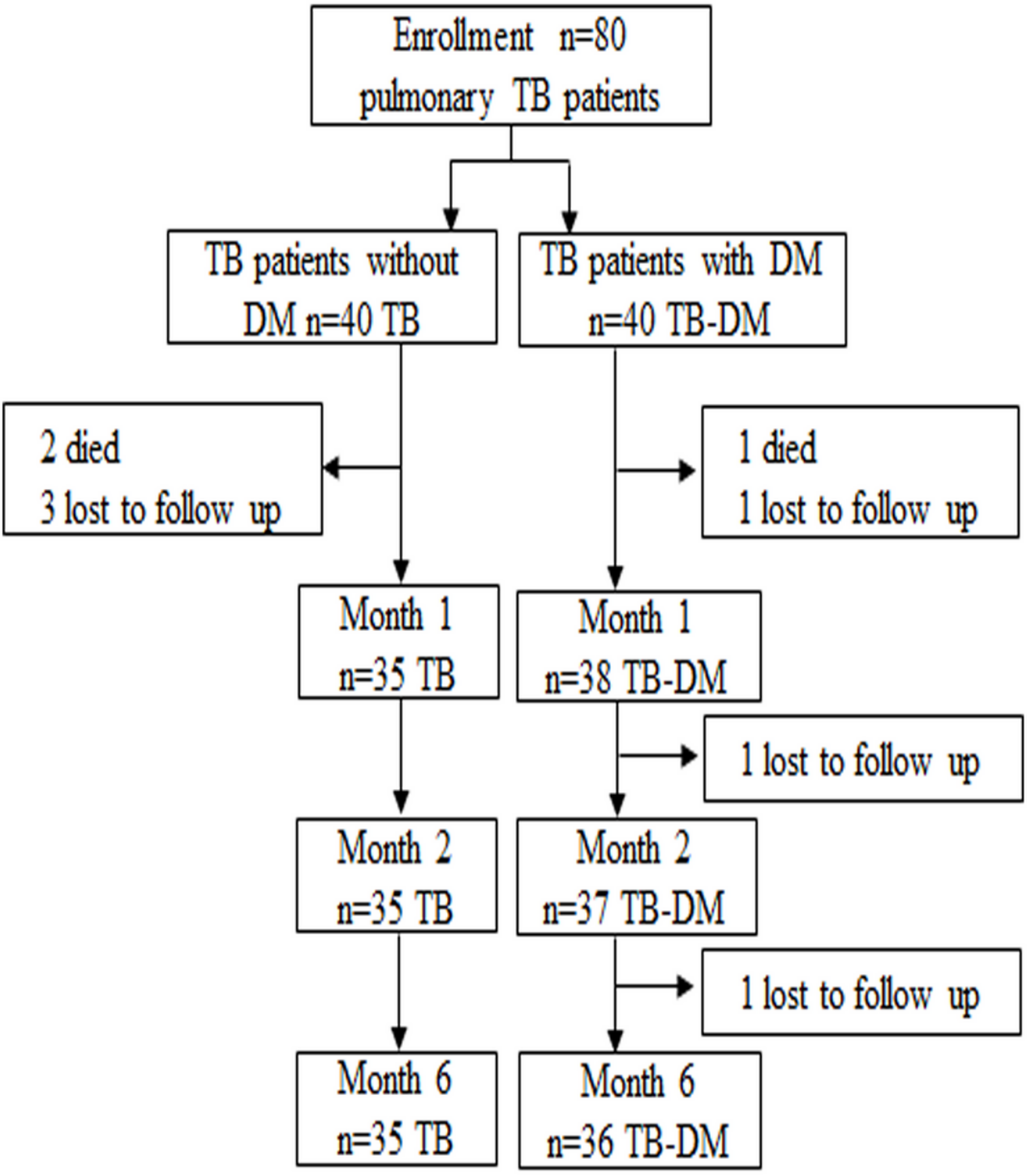

Figure 1

Flow chart illustrating enrollment and follow-up of TB and TB-DM patients.

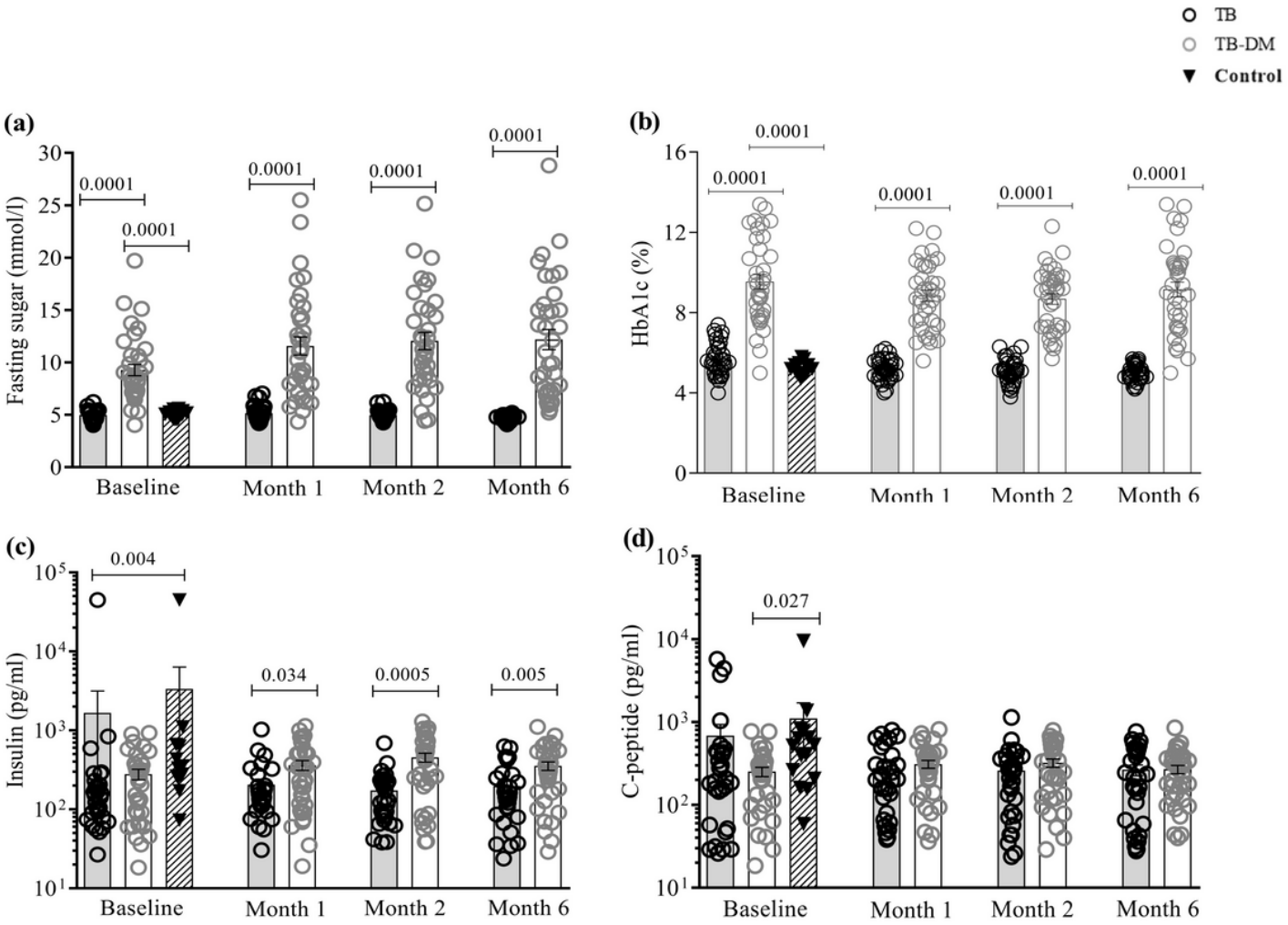

Figure 2

Glycemic markers in TB and TB-DM patients and in healthy controls. (A) Fasting blood glucose levels (mmol/L), (B) HbA1c concentrations (%), (C) plasma insulin levels (UIU), (D) plasma C-peptide (UIU) were assessed in TB (n=35) and TB-DM (n=36) patients at enrolment and after 1, 2 and 6 months of anti-TB treatment, and once in healthy controls (n=20). Data is presented as mean with 95% confidence interval. Statistical differences were calculated using adjusted multivariate regression. The regression model was adjusted for age, sex, SES score, BCG vaccination status and baseline BMI. *p < 0.05 was considered significant. BMI: body mass index; BCG: Bacillus Calmette–Guérin; HbA1c: glycosylated hemoglobin; SES: socio economic status.

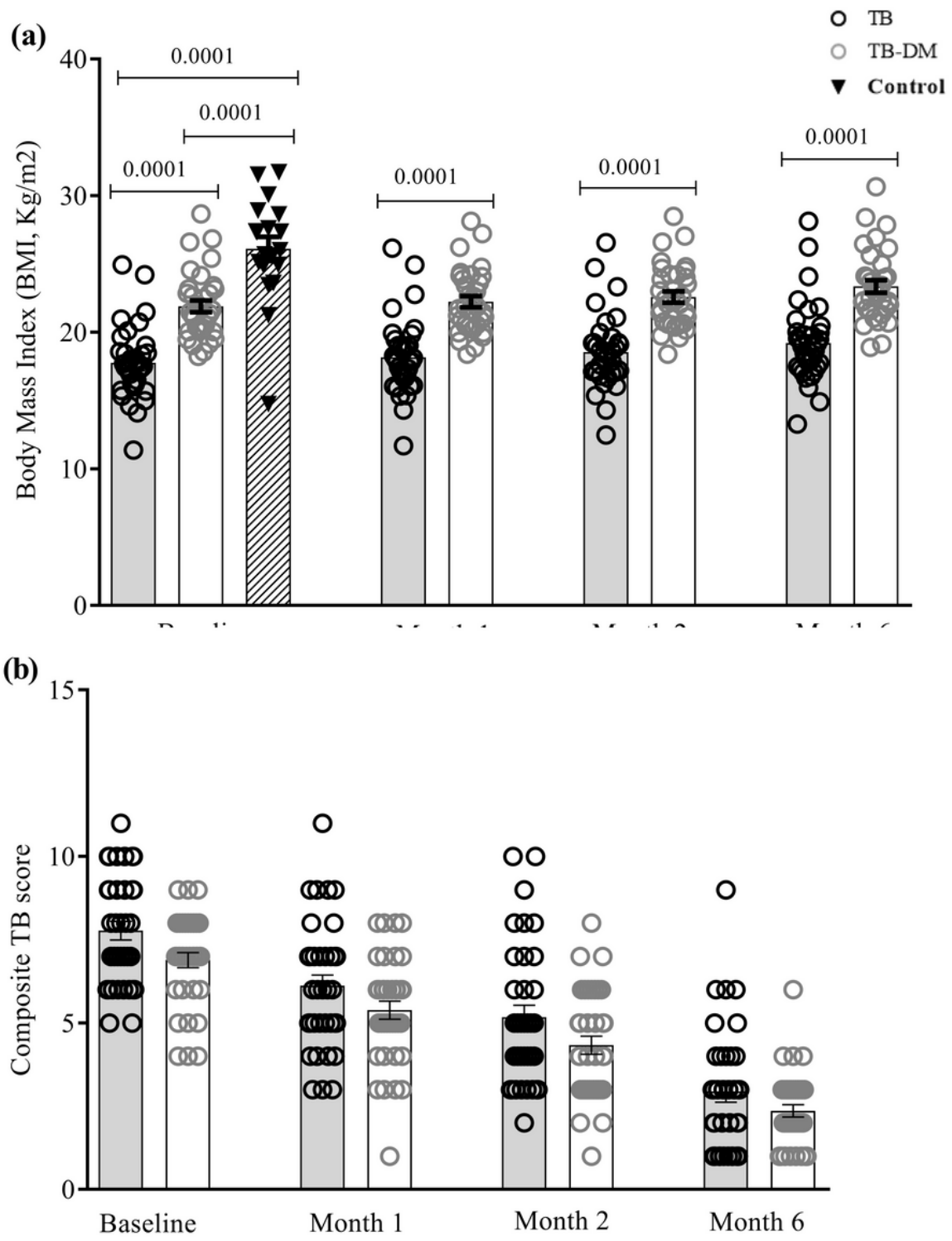

Figure 3

BMI and composite TB score in TB and TB-DM patients. (A) BMI, kg/m2 and (B) composite TB score were assessed in TB (n=35) and TB-DM (n=36) patients at enrolment and after 1, 2 and 6 months of anti-TB treatment. BMI was also assessed once in healthy controls (n=20). Data is presented as mean with 95% confidence interval. Statistical differences were calculated using adjusted multivariate regression. The regression model was adjusted for age, sex, SES score and BCG vaccination status *p < 0.05 was

considered significant. BMI: body mass index; BCG: Bacillus Calmette–Guérin; SES: socio economic status.

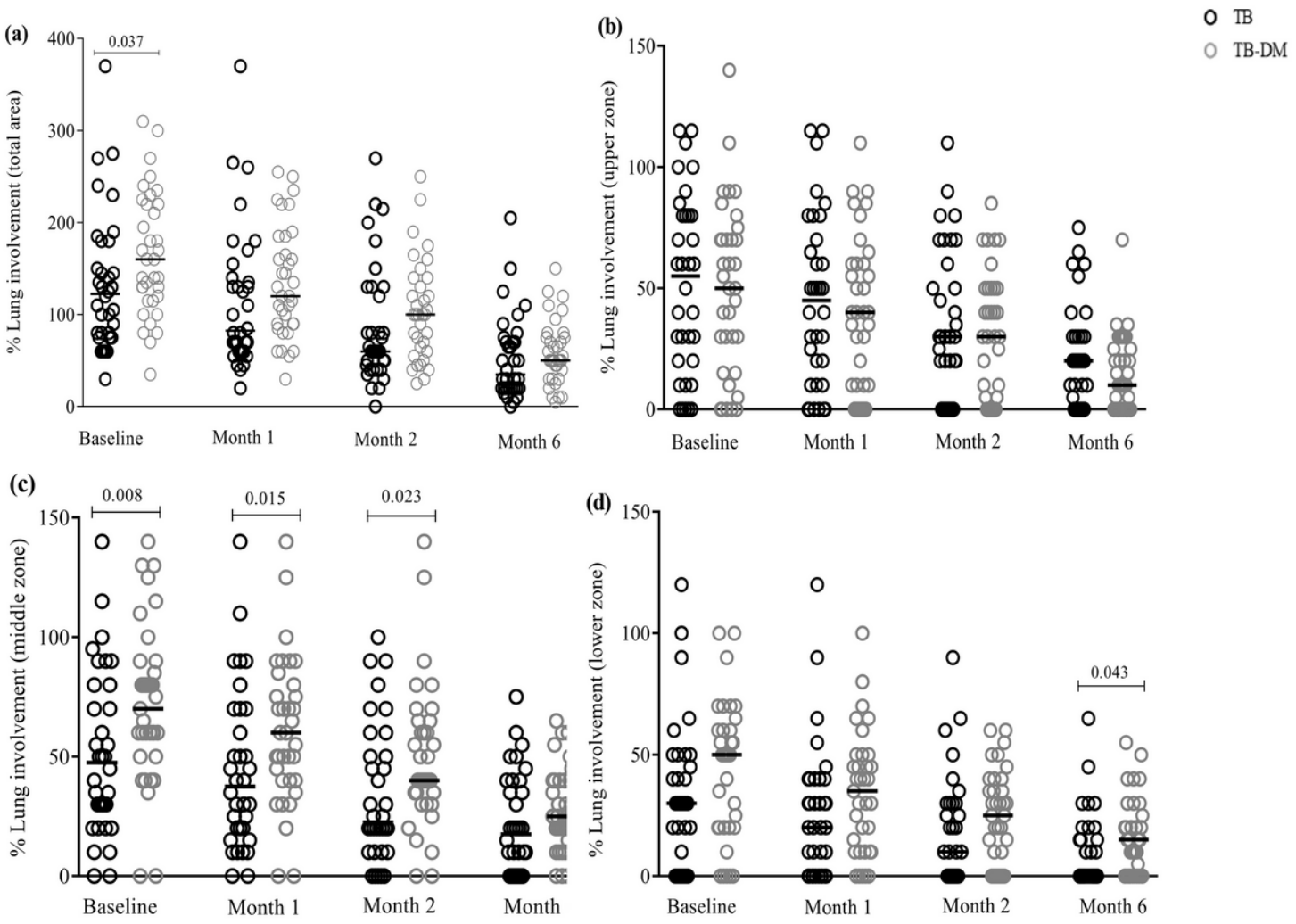

Figure 4

Chest X-ray findings in TB and TB-DM patients. (A) overall % lung involvement (upper, middle and lower zones of left and right lung combined), (B) % lung involvement in the upper zone, (C) % lung involvement in the middle zone, (D) % lung involvement in the lower zone were assessed in TB (n=35) and TB-DM (n=36) patients at enrolment and after 1, 2 and 6 months of anti-TB treatment. Data is presented as median values. Statistical differences between the TB and TB-DM group were calculated using the Mann-Whitney U-tests, and $p < 0.05$* was considered significant.

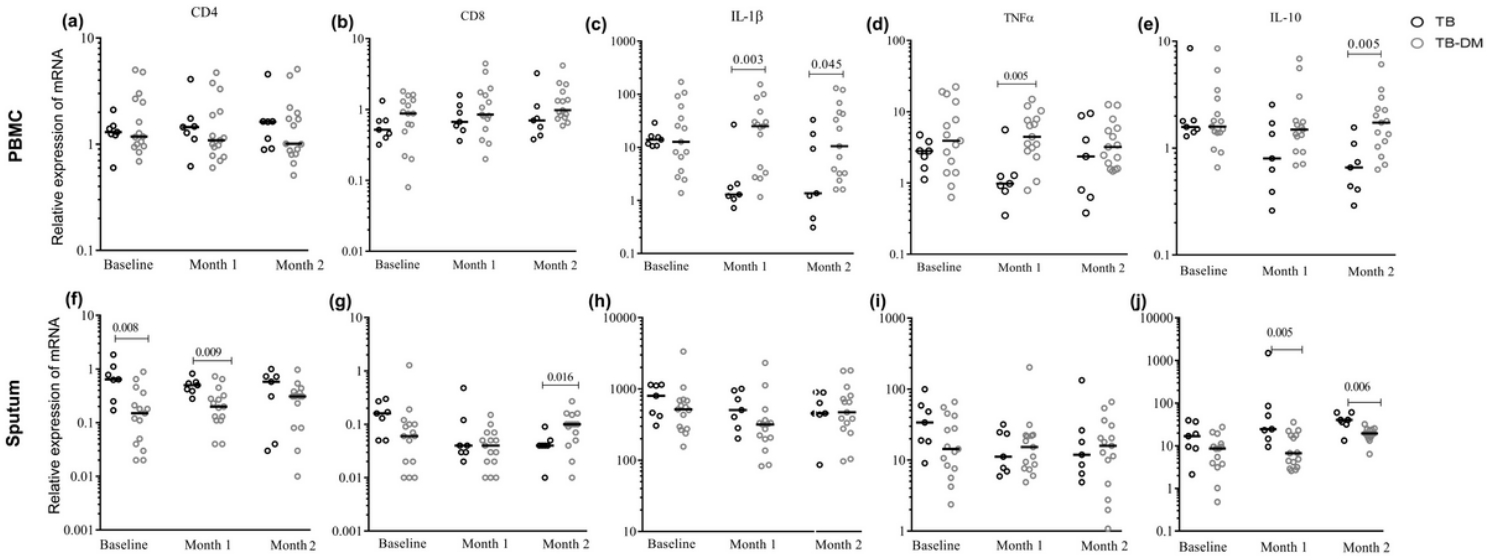

Figure 5

mRNA profiling of PBMCs and sputum cell samples from TB and TB-DM patients. Quantitative mRNA expression of CD4, CD8, IL-1, TNF- and IL-10 in PBMC (A-E) and sputum cell (F-J) samples from TB (n=7) and TB-DM (n=16) patients were analyzed at enrolment and after 1 and 2 months of anti-TB treatment. Data is presented as median values and statistical differences between the TB and TB-DM group were calculated using the Mann-Whitney U-tests, and *p < 0.05 was considered significant. Note that mRNA data is presented in dot-plot graphs with a log-scale, while the statistical analyses were performed on non-transformed data. PBMC: Peripheral blood mononuclear cells.

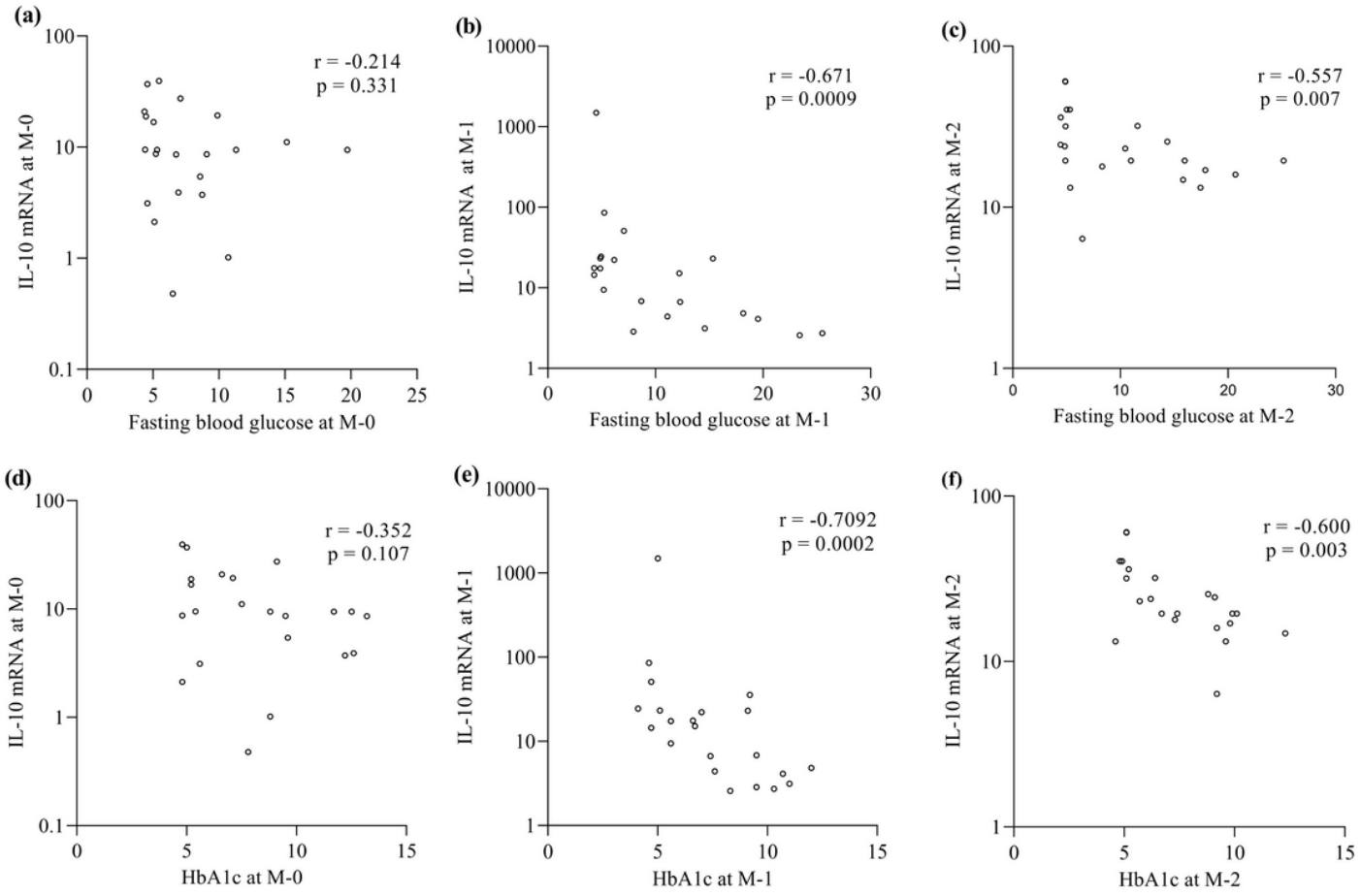

Figure 6

Correlation of sputum IL-10 transcript with fasting blood glucose and HbA1c levels. IL-10 transcript in sputum samples from TB and TB-DM patients combined (n=23) was correlated with fasting blood glucose at (A) baseline and after (B) 1 month and (C) 2 months, and HbA1c levels at (D) baseline and after (E) 1 month and (F) 2 months of anti-TB treatment. Data is presented as median and the correlation was calculated using Spearman´s correlation test. *p < 0.05 was considered significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx