Original Article

Thiamin and riboflavin status with related enzyme activities in pulmonary tuberculosis with diabetes mellitus in Shandong province of China

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Background and Objectives: Poor nutritional status is a common finding in pulmonary tuberculosis (TB) patients with and without type 2 diabetes mellitus (T2DM), thiamin (VB-1) and riboflavin (VB-2) are coenzymes important for the activation of many enzymes involved in improving nutritional status. We aimed to investigate enzymatic activities and the associations between VB-1 and VB-2, and their relations to nutritional status in TB and TB+T2DM patients. **Methods and Study Design:** This was a cross-sectional study that prospectively enrolled TB 40 patients with or without T2DM respectively from the Chest Hospital of Qingdao and 76 healthy controls with similar age and gender distributions were recruited from the medical center of the affiliated hospital of Qingdao Medical College. The erythrocyte transketolase activation coefficient (ETKac, for VB-1 deficiency), the glutathione reductase activation coefficient (EGRac, for VB-2 deficiency), and metabolic enzyme activities were analyzed. **Results:** VB-1 and VB-2 deficiency rates were higher, and enzyme activities were lower in TB and TB+T2DM relative to control group. ETKac and EGRac were negatively correlated with enzyme activities, either with body mass index (BMI), while enzyme activities were positively associated with BMI. **Conclusions:** VB-1 and VB-2 concentrations were lower in TB patients with or without T2DM relative to controls, with concomitant reductions in the activity levels of key metabolic enzymes. Significant correlations were observed between VB-1 and VB-2 concentrations and the activity of these metabolic enzymes, they all correlated with nutrition status. VB-1 and VB-2 concentrations may thus impact metabolic enzyme activity and thereby influence nutritional status.

Key Words: B vitamins, tuberculosis, type 2 diabetes mellitus, metabolic enzymes

INTRODUCTION

Pulmonary tuberculosis (TB) is a serious infectious disease caused by *Mycobacterium tuberculosis* that affected an estimated 8.9-11.0 million people globally as of 2019.[1] Type 2 diabetes mellitus (T2DM) is a metabolic disease wherein patients exhibit abnormal energy homeostasis tied to dysregulated insulin secretion, altered glucose uptake, and increased insulin resistance. As of 2014, an estimated 8.5% of the global adult population was affected by diabetes mellitus, with roughly 422 million diagnosed cases.[2] TB and T2DM exhibit similar ages of peak onset, and also exhibit synergistic etiological and pathogenic features. Indeed, in one recent report, both male and female T2DM patients were shown to be at an elevated risk of TB.[3] Restrepo BI demonstrated a three to four-fold increase in the risk of TB among diabetes mellitus patients.[4] Rates of coincident TB and T2DM have been rising in recent years, with our recent study having reported a T2DM prevalence of 6.3% among patients with active TB in rural China, with this prevalence rising to 11.9-30.8% among active TB patients in urban regions.[5]

B vitamins function as cofactors for essential metabolic enzymes such as transketolase (TKT), glutathione reductase (GR), and other mediators of fat, protein, and carbohydrate catabolism. B vitamins also play a role in dietary and gut microbiome-mediate immune modulation.[6] Thiamin (VB-1) is a cofactor important in the context of macronutrient metabolism, the active form of which is known as thiamin pyrophosphate (TPP).[7] Riboflavin

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(VB-2) is a precursor to the essential flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) cofactors that are involved in many different metabolic reactions.[8] Population-level analyses have revealed that most TB patients consume levels of macronutrients and micronutrients below the recommended daily intake values, with TB patients exhibiting mean daily VB-1 and VB-2 intake levels of 0.9 mg and 0.6 mg, respectively – levels lower than the Recommended Nutrient Intake (RNI) as 1.4 mg and 1.4 mg. Similarly, female patients exhibit mean daily VB-1 and VB-2 intake levels of 0.7 mg and 0.5 mg, respectively (RNI: 1.2 mg and 1.2 mg).[9] In our prior cohort study, we found 69.6% and 98.6% of TB patients to exhibit insufficient VB-1 and VB-2 intake, respectively.[10] Plasma VB-1 concentrations have also been found to be 75% lower in T2DM patients, while increased dietary VB-2 intake has been linked to a lower risk of T2DM incidence.[11] The relationship between VB-1 and VB-2 dietary insufficiency and comorbid TB and T2DM, however, remains to be established. TB is associated with reductions in appetite, impaired nutrient absorption, and altered metabolic activity that can ultimately contribute to wasting and poor nutritional status.[12] Lu et al found that diabetes with a lower BMI was a risk factor for developing active TB.[13] Our previous study revealed that hypoalbuminemia, anemia, and poor improvements in nutritional status were positively associated with the severity of clinical manifestations of TB.[14]

Reductions in appetite in TB patients with or without T2DM may contribute to the decreased intake of raw nutrients and B vitamins. Decreased metabolic enzyme activity may render the host unable to meet their glycolytic demands, resulting in increased blood glucose levels that can facilitate additional *M. tuberculosis* replication.[15] Malnutrition has been linked to the progression from initial M. tuberculosis infection to TB.[16] Musuenge et al found that of patients in Ouagadougou, Burkina Faso undergoing treatment for TB, 35.8% suffered from undernutrition, with T2DM being independently linked to undernutrition in this adult TB patient population.[17]

TB and T2DM patients exhibit reductions in the activity levels of many key metabolic enzymes, with reported reductions in glutathione reductase and succinate dehydrogenase activity in both TB and T2DM patients,[18-21] as well as decreased TKT and Na+/K+-adenosine triphosphatase(ATPase) activities.[22,23] However, these activity levels remain to be reported in patients with both TB and T2DM. Decreases in body mass resulting from VB-1 and VB-2 deficiencies were linked to a dramatic reduction in erythrocyte TKT activity.[24] Rock et al further found that the body weight of patients with a VB-2 deficiency was lower on average than that of patients with adequate VB-2 intake.[25] Potential synergistic interactions between metabolic enzymes and B vitamins in TB patients are poorly understood, particularly in the context of comorbid T2DM. To that end, the present study was conducted to explore VB-1 and VB-2 deficiency among TB patients with and without T2DM and to elucidate the correlations between these B vitamins concentrations and metabolic enzyme activity levels. Together, these data may provide evidence regarding the ability of VB-1 and VB-2 to improve patient nutritional status via promoting enzymatic activity, and may further highlight new approaches to treating poor nutritional status in TB patients with or without T2DM.

METHODS

Subjects

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee of Medicine of Qingdao center of disease control and prevention (2009, NO.5). The clinical trial registration number was ChiCTR-OCC-10000994.

This was a cross-sectional study that prospectively enrolled 40 patients with TB and T2DM (TB+T2DM) from the Chest Hospital of Qingdao. These patients exhibited positive M. tuberculosis sputum cultures and positive chest X-ray results, as well as a fasting plasma glucose ≥7.0 mmol/l (mM) or a random blood sugar level >11.1 mM. In addition, 40 TB patients without T2DM were recruited from this same hospital, while 76 healthy controls with similar age and gender distributions were recruited from the medical center of the affiliated hospital of Qingdao Medical College. Patients were excluded from this study if they were diagnosed with type 1 diabetes mellitus (T1DM), miliary TB, non-tuberculous mycobacteria (NTM), or human immunodeficiency virus co-infection. In addition, patients with any serious comorbidities or who have previously been treated with anti-TB drugs were excluded.

Subjects were weighed barefoot with minimum clothing using an electronic weighing scale, body weight was recorded to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using stadiometer, the body mass index (BMI) was calculated as Weight (kg)/ height^2 (m).

Erythrocyte metabolic enzyme measurements

A drop of blood was collected from the finger to measure erythrocyte metabolic enzymes. TKT activity was measured based upon the enzymatically measured erythrocyte transketolase activation coefficient (ETKac). The percentage of diphosphothiamine and the TPP effect were determined using a previously published protocol with the TPP effect being determined as a percentage of ETKac where 0–15%, 15-25%, and >25% were considered to indicate normal, marginally deficient, and deficient VB-1 concentrations. [26]

The activity of glutathione reductase (GR) was determined based upon the erythrocyte glutathione reductase activation coefficient (EGRac), which was the ratio of stimulated flavin-adenine dinucleotide activity to unstimulated erythrocyte GR activity.[27] EGRac levels of <1.2 were considered indicative of adequate VB-2 concentrations, while values of 1.2–1.5 were indicative of normal VB-2 concentrations, values of 1.51–1.8 were indicative of a low VB-2 concentrations, and values >1.80 were consistent with VB-2 deficiency. Higher EGRac values coincide with progressive reductions in GR activity.[27]

Erythrocyte Na+/K+-ATPase activity (A070-6, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was assessed with a spectrophotometer at 540 nm.

Plasma metabolic enzyme analyses

Plasma pyruvic acid kinase (PK) (A076-1), succinate

dehydrogenase (SDH) (A022), and malate dehydrogenase (MDH) (A021-1) levels were assessed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on provided directions.

Statistical analysis

A two-sided alpha value of 5% and a power level of 80% was for sample size estimation purposes. The Kolmogorov-Smirnov test was used to assess the normality of data distributions, with non-normally distributed data for continuous variables being subjected to $\log_{10}$ transformation prior to parametric analyses. Normally distributed data are given as means±SD, whereas $\log_{10}$-transformed data are reported as geometric means. Data were compared via two-way ANOVAs or non-parametric tests when normally and non-normally distributed, respectively. Pearson correlation coefficients were used to estimate relationships between transformed variables. Associations among different parameters were additionally evaluated via multivariate linear regression analysis. SPSS 22.0 was used for all statistical testing, with a p-value <0.05 as the threshold of significance.

RESULTS

Table 1 compiles biochemical and anthropometric data for patients in the three study groups. The BMI of patients in the TB (21.1±4.07 kg/m^2) and TB+T2DM (21.7±3.46 kg/m^2) groups were lower on average than that of control study participants (22.9±2.14 kg/m^2). Patients in the TB+T2DM group exhibited higher serum glucose levels as compared to TB patients and controls (9.51±2.29 vs 5.81±2.60 mM, 9.51±2.29 vs 5.19±0.59 mM, respectively, p<0.05). Serum HDL-cholesterol, protein, uric acid, and creatinine levels were higher in control patients relative to individuals in the TB and TB+T2DM groups (p<0.05), whereas the opposite trend was observed for serum alanine aminotransferase and aspartate aminotransferase levels in these control individuals relative to the TB and TB+T2DM groups (p<0.05).

VB-1 nutritional status for these patients is summarized in Figure 1. The percentage of patients with normal VB-1 levels in the TB and TB+T2DM groups was lower than in the control group (57.8% and 57.1% vs 82.1% respectively, p<0.05). The incidence of VB-1 marginal deficiency was higher in the TB group relative to the control group (17.8% vs. 6.9%, p<0.05). The percentage of VB-1 deficiency in the TB+T2DM group was higher than in the TB group, and both were significantly higher than that in the control group (32.1% and 24.4% vs 11.0%, p<0.05). VB-2 nutritional status findings are summarized in Figure 2. There were fewer patients in the TB+T2DM group that exhibited normal VB-2 concentrations relative to the control group (62.1% vs 80.6%, p<0.05) and the TB group. VB-2 deficiency was more prevalent in the TB+T2DM group relative to the TB group, and both groups exhibited significantly higher prevalence relative to the control group (20.7% and 14.3% vs 4.4% respectively, p<0.05). The percentage of VB-2 marginal deficiency did not differ significantly among these groups, but the TB+T2DM groups exhibited higher percentages than did the TB group.

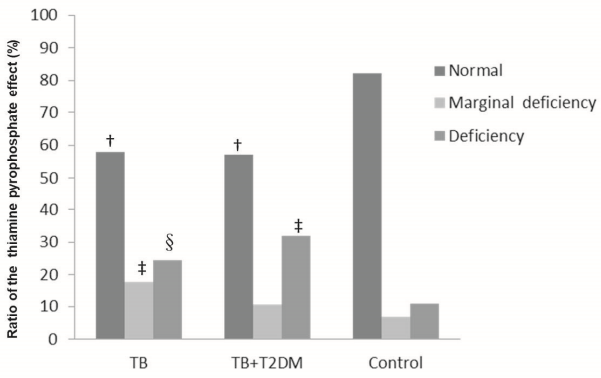

Figure 1. VB-1 nutritional status in different patient cohorts. †Comparison of normal VB-1 status with the control group, p<0.05; ‡Comparison of VB-1 deficiency status with the control group, p<0.05; §Comparison of VB-1 deficiency status with the control group, p<0.05.

Table 2 summarizes the metabolic enzyme activity levels in different groups. ETKac was highest in the TB+T2DM group (41.9±8.53% vs 38.1±9.08% and

Table 1. Study subject characteristics (N=156)

	TB	TB+ T2DM	Control
N (male/female)	40 (31/9)	40 (35/5)	76 (49/27)
Age (years)	57.6±14.0	58.2±12.4	61.6±13.6
BMI (kg/m^2)	21.1±4.07	21.7±3.46	22.9±2.14 †,‡
Glucose (mM)	5.81±2.60	9.51±2.29†,§	5.19±0.59
Cholesterol (mM)	4.44±1.21§	4.62±1.32	4.90±0.86
Triglycerides (mM)	1.03±0.38‡	1.24±0.60	1.15±0.58
VLDL-cholesterol (mM)	2.50±0.77	2.28±0.65§	2.64±0.66
HDL-cholesterol (mM)	1.13±0.37	1.21±0.33	1.57±0.36†,‡
Total protein (g/L)	62.4±7.85	63.4±7.52	75.9±4.24†,‡
Albumin (g/L)	38.4±6.63	37.6±5.44	43.5±1.99†,‡
Alanine aminotransferase (U/L)	28.8±11.1	30.7±11.6	19.9±7.32†,‡
Aspartate aminotransferase (U/L)	25.6±5.86	26.9±8.47	21.1±5.74†,‡
Uric acid (mM)	289±29.1	295±21.3	400±83.4†,‡
Serum creatinine (mM)	54.8±14.5	48.3±15.7	78.5±18.3†,‡

BMI: body mass index; VLDL: very-low-density lipoprotein; HDL: high-density lipoprotein.
Data are means±SD.
† Compared with TB, p<0.05
‡ Compared with TB+T2DM, p<0.05
§ Compared with Control, p<0.05.

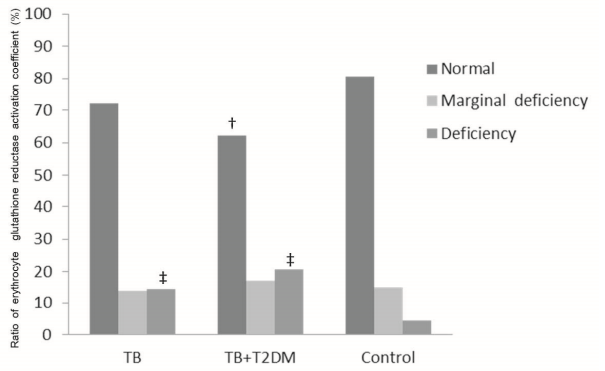

Figure 2. VB-2 nutritional status in the indicated patient cohorts. †Comparison of normal VB-2 status with the control group, $p<0.05$; ‡Comparison of VB-2 marginal deficiency status with the control group, $p<0.05$.

8.00±2.78% respectively), with the same also being true for EGRac (1.24±0.61% vs 1.23±0.27% and 0.86±0.55% respectively). Conversely, Na+-K+-ATPase levels were lower among TB+T2DM patients relative to TB patients ($p<0.05$), and both groups were lower relative to controls (13.0±2.31 U/mL and 18.5±1.38 U/mL vs 28.4±2.11 U/mL respectively, $p<0.05$). TB+T2DM patients also exhibited lower PK levels relative to individuals in the TB and control groups (23.7±3.61 U/g of protein vs. 30.0±5.32 U/g of protein and 30.4±9.66 U/g of protein, respectively, $p<0.05$), while SDH levels were lower in the TB and TB+T2DM groups as compared to the control group (10.6±1.50 U/mL and 11.7±1.52 U/mL vs. 20.6±5.29 U/mL respectively, $p<0.05$). MDH levels were significantly lower in the TB+T2DM group relative to the control group (0.63±0.17 vs. 0.78±0.26 U/mL, $p<0.05$) and the TB group.

Correlations between metabolic enzyme activity levels and VB-1 nutritional status (ETKac) are shown in Table 3. EGRac was positively correlated with ETKac in the TB and TB+T2DM groups (r=0.149, r=0.194, respectively, $p<0.05$). Na+-K+-ATPase, PK, and SDH levels were negatively correlated with ETKac in both the TB and TB+T2DM groups ($p<0.05$). MDH was negatively correlated with ETKac in the TB+T2DM group ($p<0.05$). Correlations between metabolic enzyme activity levels and VB-2 nutritional status (EGRac) are shown in Table 4. Na+-K+-ATPase, PK, MDH were negatively correlated with EGRac in both groups ($p<0.05$).

The results of multivariate linear regression analyses examining the relationship between BMI and other factors are presented in Table 5. In the TB group, BMI was negatively correlated with ETKac and EGRac (-5.064, $p=0.032$, and -2.625, $p=0.049$) while it was positively associated with PK and SDH (2.124, p=0.047, and 5.346, $p=0.039$). In the TB+T2DM group, BMI was similarly negatively correlated with ETKac and EGRac (-4.302, $p=0.036$, and -2.877, $p=0.048$) but positively associated with PK and MDH (2.317, $p=0.041$, and 5.424, $p=0.025$).

DISCUSSION

Patients with TB often exhibit a reduced appetite together with digestive and absorption disorders linked to impaired nutrient intake. In this study, we found that the average BMI, protein levels, and albumin levels measured in patients with TB or TB+T2DM were lower than those in healthy controls, consistent with poorer nutritional status in these individuals, in line with prior evidence.[28,29] *M. tuberculosis* is known to preferentially acquire and metabolize host-derived lipids, which fuel bacterial growth and persistent pathogenesis.[30] T2DM is linked to abnormal lipid metabolism, resulting in elevated cholesterol and triglyceride levels in affected patients. Vrieling et al. observed a high level of heterogeneity in lipid metabolism among individuals in their TB+DM patient population.[31] Here, we found that patients in the TB+T2DM group exhibited higher cholesterol and triglyceride levels than did patients in the TB group, whereas HDL levels were lower in both TB and TB+T2DM patients relative to healthy controls, potentially owing to the fact that inflammatory processes can reduce lecithin cholesterol acyltransferase activity.[32]

Mirlohi et al. found that following M. tuberculosis infection, ALT and AST levels rise significantly, and so was in DM alone.[33] Consistently, we observed increased ALT and AST levels in TB, as we have observed previously,[34] and we further found that TB+T2DM exhibited the highest levels for both of these parameters, potentially as a consequence of liver damage induced by TB in the context of comorbid T2DM.[35] Creatine can reportedly accelerate amino acid absorption and promote protein synthesis.[36] Here, we found serum creatinine levels to be lower for patients in the TB and TB+T2DM groups relative to controls, with the lowest levels in the TB+T2DM group, potentially because our samples were derived from primary TB patients before drug treatment and these individuals exhibited insufficient protein intake, suggesting that protein metabolism may be more deregulated in

Table 2. Metabolic enzyme activities in different groups

Indicator	TB	TB+T2DM	Control
ETKac (%)	41.9±8.53	38.1±9.08	8.00±2.78†,‡
EGRac (%)	1.24±0.61	1.23±0.27	0.86±0.55†,‡
Na^+-K^+-ATPase (U/mL)	18.5±1.38	13.0±2.31†	28.4±2.11†,‡
PK (U/g of protein)	30.0±5.32	23.7±3.61†,§	30.4±9.66
SDH (U/mL)	10.6±1.50	11.7±1.52	20.6±5.29†,‡
MDH (U/mL)	0.75±0.38	0.63±0.17§	0.78±0.26

Results are means±SD.
ETKac: erythrocyte transketolase activation coefficient; EGRac: erythrocyte glutathione reductase activation coefficient; PK: pyruvate kinase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase.
† Compared with TB, $p<0.05$;
‡ Compared with TB+T2DM, $p<0.05$;
§ Compared with Control, $p<0.05$.

Table 3. Correlations between metabolic enzymes and ETKac in different groups

	TB	TB+T2DM
EGRac	0.149*	0.194*
Na^+-K^+-ATPase	-0.218*	-0.176*
PK	-0.187*	-0.144*
SDH	-0.479*	-0.519*
MDH	-0.027	-0.231*

EGRac: erythrocyte glutathione reductase activation coefficient; PK: pyruvate kinase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase.
*$p<0.05$.

Table 4. Correlations between metabolic enzymes and EGRac in different groups

	TB	TB+T2DM
ETKac	0.149*	0.194*
Na^+-K^+-ATPase	-0.237*	-0.163*
PK	-0.227*	-0.135
SDH	-0.128	-0.122
MDH	-0.229*	-0.269*

ETKac: erythrocyte transketolase activation coefficient; PK: pyruvate kinase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase.
*$p<0.05$.

Table 5. Relationship of BMI with metabolic enzymes, VB-1 and VB-2 status in different groups

t	TB		TB+T2DM	
	β coefficients	*p*	β coefficients	*p*
ETKac	-5.064	0.032*	-4.302	0.036*
EGRac	-2.625	0.049*	-2.877	0.048*
Na^+-K^+-ATPase	-0.028	0.777	0.837	0.225
PK	2.124	0.047*	2.317	0.041*
SDH	5.346	0.039*	1.028	0.137
MDH	0.089	0.854	5.424	0.025*

ETKac: erythrocyte transketolase activation coefficient; EGRac: erythrocyte glutathione reductase activation coefficient; PK: pyruvate kinase; SDH: succinate dehydrogenase; MDH: malate dehydrogenase.
*$p<0.05$.

TB+T2DM patients.

Many B vitamins are essential coenzymes involved in cellular metabolic processes. Snášel et al. demonstrated that B vitamins can potently inhibit M. tuberculosis pyruvate kinase activity,[37] and as such, VB deficiency may render patients more susceptible to TB.[9] Our results further confirmed that VB-1 and VB-2 deficiencies were more common in TB patients relative to controls, as the percentages of VB-1 and VB-2 sufficiency in the TB+T2DM group were significantly lower than those in the TB group. T2DM can also influence B vitamin-related biological processes. For example, Pácal et al. observed an 8-fold increase in renal VB-1 clearance in an experimental DM model system,[38] potentially contributing to the abnormally low plasma VB-1 concentrations observed in T2DM patients. VB-2 supplementation has also been shown to be associated with impaired glucose metabolism.[39] We previously detected reduced VB-1 and VB-2 intake in TB patients,[10] and here we observed a higher frequency of VB-1 and VB-2 deficiency in the TB+T2DM group relative to the TB group, suggesting that in addition to the increased urinary flow and impaired reabsorption in these patients leading to increased B vitamin excretion,[40] low B vitamin intake and general B vitamin deficiency in TB+T2DM patients is also due to the improper dietary patterns among individuals in Shandong province, who exhibited an excessive ratio of carbohydrates and the increased metabolic impairment of glucose. Efforts to increase the intake and normalize associated metabolic activities and nutritional status using foods rich in VB-1 and VB-2 such as poultry, fish, and fresh vegetables should be considered for daily intake, as we reported previously.[41]

TKT is a VB-1-dependent enzyme that is responsible for catalyzing carbohydrate transformation in the pentose phosphate pathway. Michalak et al. observed a reduction in basal TKT activity in patients with diabetic neuropathy with VB-1 deficiency.[22] We found that TKT activities, which were negatively correlated with ETKac, were significantly lower in TB and TB+T2DM patients relative to controls, indicating a negative correlation between TKT activity VB-1 concentrations, and disease risk.[42] GR plays a role in the TCA cycle and promotes protein, fat, and glucose metabolism,[43] and can be measured to assess VB-2 concentrations. In prior reports, neutrophil GR activity was found to be significantly reduced in TB patients relative to normal controls.[18] Kumawat et al. determined that GR activity and erythrocyte gene expression were reduced in T2DM patients relative to healthy individuals.[19] We additionally found that erythrocyte GR activity, which was negatively correlated with EGRac, was significantly reduced in the TB and TB+T2DM groups relative

to control individuals, consistent with prior reports. This may be attributable to decreased food intake, impaired glucose metabolism, and nutrient malabsorption, resulting in reduced levels of cytoplasmic nicotinamide adenine dinucleotide phosphate (NADPH) and GR output.[44]

Na^+-K^+-ATPase can hydrolyze ATP to facilitate the transport of Na^+, K^+, carbohydrate and amino acids. Na^+-K^+-ATPase expression and activity levels are closely tied to T2DM and related metabolic disorders.[45] In population-based studies, erythrocyte Na^+-K^+-ATPase activity was found to be lower in DM patients relative to healthy individuals.[23] We observed reductions in Na^+-K^+-ATPase activity in the TB+T2DM group relative to TB, which can further exacerbate the effects of TB on Na^+-K^+-ATPase, potentially through mechanisms associated with increased oxidative/nitrosative stress and further glutathione depletion in T2DM.[46]

PK plays a role in the sugar metabolism pathway in the context of energy production.[47] Here, we observed significantly reduced PK activity in the TB+T2DM group relative to TB group, consistent with a robust negative correlation between PK activity and blood glucose levels in DM as has previously been reported.[48] This may be attributable to reduced insulin availability and/or post-translational modification of this enzyme.[49]

SDH oxidizes succinate to yield fumarate in the context of the TCA cycle, in addition to oxidizing ubiquinone to ubiquinol in the mitochondrial electron transport chain.[50] Clinical analyses have also found that SDH activity is inhibited in peripheral blood lymphocytes from patients with TB.[51] Mitochondrial SDH activity in the pancreas is also significantly reduced in myocardial and pancreatic tissues from diabetic rats,[20] and mitochondrial SDH activity levels in the skeletal muscle of T2DM patients are decreases relative to those in healthy individuals.[21] In this study, we observed significantly lower SDH activity levels in the TB and TB+T2DM groups relative to controls, potentially due to the diminution, during the course of the tuberculous infection, of some unidentified substance which was present in the normal tissue and influenced the SDH activity.[52] This may be linked to the impact of glycerol, alanine, and serine on the TCA cycle, given that these are central metabolites associated with diabetes.[53]

MDHs are responsible for catalyzing oxaloacetate and malate interconversion in the context of the oxidation/reduction of dinucleotide coenzymes.[54] Lin et al found that T2DM was associated with a reduction in MDH activity.[53] Ryder et al determined that MDH levels were decreased by 25% in T2DM patients.[55] We further found that SDH activity levels were lower among TB+T2DM patients relative to TB with no significant differences being observed. This suggests that T2DM alone may impact MDH activity, potentially through mechanisms linked to hypozincaemia and depressed energy metabolism in T2DM.[56]

Correlation analyses indicated a positive correlation between ETKac and EGRac levels, in line with a previous report published by Hrubša et al. suggesting that VB-1 and VB-2 concentrations mutually influence one another in a synergistic manner. [57] Hoyumpa et al. found that impaired VB-1 movement out of enterocytes is correlated with reduced Na^+-K^+-ATPase activity,[58] while VB-2 exit from enterocytes is Na^+-dependent and directly coupled to Na^+-K^+-ATPase-mediated ATP hydrolysis, further supporting a link between Na+-K+-ATPase activity and B vitamin transport.[59] One study reported that total PK activity was related to VB-1 deficiencies.[60] Staal et al found that daily VB-2 administration for 6 months was associated with the restoration of normal GR and PK activity.[61] Herein, we found that PK activity was positively correlated with VB-1 and VB-2 in TB and TB+T2DM groups, consistent with the reports above. Bubber et al. determined that VB-1 deficiency can induce oxidative stress and increase reactive oxygen species concentrations,[62] ultimately driving increased activity of TCA cycle enzymes including MDH and SDH. Tang et al. reported that VB-2 deficiency can also reduce the activity of key TCA cycle enzymes.[63] We found that SDH and MDH levels in TB patients with or without T2DM were significantly positively correlated with VB-1 levels, while MDH levels were also positively correlated with VB-2 levels in both TB patient groups, suggesting a potential link to TCA cycle activity.[63] We also found that B vitamin levels and enzyme activities were positively correlated with BMI, which was used as an indicator of general nutritional status, suggesting that improvements in nutritional status and increases in BMI may correspond to increases in VB-1 and VB-2 levels and enzymes activities. Correlations between nutritional status and the activity levels of these enzymes have not been previously reported, and it remains unknown as to whether B vitamins can impact enzyme activity levels to improve nutritional status in TB patients, particularly in the context of comorbid T2DM. However, our findings suggest that the nutritional status of TB patients with and without T2DM may be associated with VB-1 and VB-2 levels and with the activity of B vitamin-related metabolic enzymes.

Our study focused on TB+T2DM patients, a group which are relevant to the unique medical situation in China where these two comorbid diseases constitute a double-burden in some areas and populations. Our study provides important data that can guide current understanding of VB-related nutritional status and enzyme activities in those with TB, particularly among patients that are also affected by T2DM. We have conducted randomized clinical trials comparing Vitamin A and/or Vitamin D supplementation outcomes in active pulmonary TB patients with DM, and these data suggest that B vitamin supplementation also warrants further consideration.[64-66] Limitations of this study include our relatively small sample size and the limited number of female patients, precluding us from investigating the relationships between sex, VB status, and enzyme activity levels. As we focused on older patients, we were also unable to conduct age-based subgroup analyses. Further large-scale research will be essential to validate our findings.

Conclusion

In summary, the results of this study offer novel insight regarding the relationship between poorer nutritional status (as indicated by a lower BMI), VB-1 and VB-2 deficiencies, and reduced metabolic enzyme activity levels in TB patients with and without T2DM. In light of these

results, we conclude that abnormal B vitamin levels and metabolic enzyme dysregulation may be linked to the incidence of poor nutritional status, with VB-1 and VB-2 potentially affecting enzyme activity levels given the observed positive correlations in the present analysis. As such, B vitamin supplementation including through daily food intake may be a viable approach to improving the nutritional status of individuals with TB and/or T2DM owing to consequent increases in metabolic enzyme activity levels. However, further research will be needed to reaffirm these findings and to evaluate the underlying molecular mechanisms, particularly as patients with comorbid TB and T2DM may exhibit more complex pathogenic findings relative to those observed in individuals with TB alone.

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AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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