A Double Burden of Tuberculosis and Diabetes Mellitus and the Role of Vitamin D Deficiency

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Contents

Abstract

Tuberculosis remains a major global health challenge, particularly in low-to-middle income countries such as China. At the same time, the country is facing a rapidly increasing diabetes incidence over the last 10 years. Diabetes aggravates the tuberculosis epidemic which poses a serious challenge in public health. In recent years, the high prevalence of vitamin D deficiency represents a global health problem, which is also associated with the risk of diabetes, and tuberculosis. Therefore, we carried out this study aimed to investigate the epidemiology of co-occurrence of tuberculosis and diabetes and to elucidate the role of vitamin D deficiency.

In a rural area setting in Linyi, Shandong, China, 6382 active tuberculosis patients, together with 6674 non-tuberculosis controls were screened for diabetes. The prevalence of diabetes in tuberculosis was 6.3%, nearly 35% higher than controls. Tuberculosis was independently associated with about three times higher risk of having diabetes. Hyperglycemia was found to be associated with a nearly two-fold higher probability of patient delay of tuberculosis in a randomly selected subgroup from this study. Compared with the highest quartile, subjects in the lowest quartile of serum 25(OH)D had a more than four-fold and two-fold increased risk of having tuberculosis, and concurrent tuberculosis and diabetes. Subsequently, a city level hospital-based study was carried out. A high prevalence of vitamin D deficiency of nearly 80% was observed in tuberculosis patients, and serum 25(OH)D was observed to be inversely associated with TNF-α, while positively associated with leptin, indicating a possible immune-modulatory mechanism of vitamin D. In the intervention trial of vitamin D3 (800IU/d), 358 newly diagnosed tuberculosis patients were included and more severe signs and symptoms at presentation were observed in those with diabetes.

Vitamin D showed a favourable adjunctive effect on clinical manifestations in this subgroup, while no similar effects were found in patients with tuberculosis only.

In conclusion, active tuberculosis patients had an increased risk of having diabetes, as well as prevalent hypovitaminosis D. Vitamin D showed an adjunctive effect in the therapy, i.e., improvement of tuberculosis clinical manifestations in patients with combined diabetes and tuberculosis. Our study provides possible etiological clues for the combined diseases, and the possible role of vitamin D deficiency. We recommend bidirectional screening for tuberculosis and diabetes, especially early detection of tuberculosis in diabetes patients; and health education to increase awareness of the double burden. In addition, vitamin D supplementation may benefit tuberculosis patients in general. Moreover, extra vitamin D may benefit the general population especially in a situation of a heavy tuberculosis burden combined with prevalent vitamin D deficiency. We suggest future studies to address long-term treatment outcomes of patients with the combined diseases and to define certain markers to set up a prognostic model. Also, longitudinal studies to verify the role of vitamin D deficiency in the double burden, and confirmative trials on the effect of vitamin D supplementation are needed.

1 General introduction

Tuberculosis in China

Tuberculosis, which is caused by *Mycobacterium tuberculosis*, continues to be an important global health challenge, with an estimated 9 million incident cases worldwide each year. Although a downward trend of tuberculosis incidence has been observed from 2000 to 2015, China still accounts for nearly 17% of the world's tuberculosis burden, with approximately 0.9 million new cases in 2016 [1].

Several factors are known to increase the risk of active tuberculosis, including age, malnutrition, underweight, unhealthy living habits like smoking and alcoholism, and comorbidities like human immunodeficiency virus (HIV) infection, and diabetes mellitus.

Diabetes Mellitus in China

In recent years, the global incidence of type 2 diabetes mellitus has increased significantly, rising from 4.7% in 1980 to 8.5% in 2014 in the adult population [2]. It is estimated that the number of people living with diabetes will rise from 382 million (in 2013) to 592 million by 2035 [3]. About 85-95%

of the global prevalence of diabetes is attributed to type 2 diabetes mellitus, which results from the body's failure to adequately respond to insulin. Genetics, age, family history of diabetes, unhealthy nutrition and physical inactivity are the main factors that are associated with the risk of type 2 diabetes. Also, it is widely accepted that excess weight is closely related to increased risk of developing type 2 diabetes [4] [5]. In comparison to women with normal BMI, overweight, obese (30 ≤ BMI < 39.99), and severely obese (BMI ≥ 40) women face increased risk of developing type 2 diabetes with 7.6%, 20.1% and 38.8% greater risk, respectively [4]. Moreover, BMI above normal weight levels has been observed to be associated with an increased risk of complications of diabetes mellitus [6]. However, low BMI may also be related with adverse effects on health. Accumulating evidence in recent years has indicated the potential adverse influence of underweight on cardiometabolic milieu. A U-shaped relationship was observed between BMI and all-cause mortality [7], and low BMI was associated with adverse outcomes of coronary heart disease in an Asian patient population [8]. A large-scale cohort study in Japan showed that underweight may be associated with the risk of type 2 diabetes among older adults aged 60–79 years [9].

In China, due to industrialization, urbanization, extended life expectancy and lifestyle changes, the prevalence of diabetes has increased especially rapidly over the last 10 years [10]. The International Diabetes Foundation Diabetes Atlas estimated that in 2017 the number of persons with diabetes in China was 114 million, equating to nearly a quarter of cases worldwide.

The Double Burden of Diabetes Mellitus and Tuberculosis

About one-third of the world's population is infected with M. tuberculosis. Most of them will develop latent infection, and only nearly 5~10% of them will progress to the active form of the disease, with approximately 9 million

individuals developing tuberculosis each year [1]. When the integrity of the immune system is compromised, as is the case for instance in HIV infection and diabetes, the risk increases significantly.

Diabetes is a well-known risk factor for tuberculosis. Prior to the insulin era, diabetes was a great threat to health and the leading cause of death was tuberculosis. Due to the rapidly increasing prevalence of diabetes worldwide

PERCENTAGE OF TB CASES ATTRIBUTABLE TO DIABETES, 2011 (AGE 20 – 79)

<2
2-4
4-6
6-8
8-10
>10

SOURCE: International Diabetes Federation World Diabetes Atlas

Figure 1-1   The percentage of tuberculosis cases attributable to diabetes in the world (2011)

in recent years, the correlation between tuberculosis and diabetes and its implication to public health is attracting renewed and intensive attention.

Diabetes is estimated to triple the risk of active tuberculosis [11], whereas about 10~15% of cases is attributable to diabetes [1; 12] [13; 14] (Figure 1-1). These findings suggest that diabetes is a moderate to strong risk factor for active tuberculosis.

Although there has been some dispute with respect to cause and consequence, it is widely accepted that diabetes usually comes first in the occurrence of combined disease. Whether tuberculosis might in turn increase the risk of diabetes is still inconclusive. Some studies have pointed out that tuberculosis can cause temporary elevation of blood glucose by fever and toxin produced by *M. tuberculosis*. Furthermore, anti-tuberculosis drug treatment may be harmful to islet cells and hamper insulin secretion. However, this may also be the result of a stress response and even resolve with treatment.

Currently, 80% of global diabetes mellitus burden is in low-and middle-income countries. Six of them (India, China, Brazil, Indonesia, Pakistan and The Russian Federation) also have a high tuberculosis burden [1].

Due to its immuno-compromising effect, diabetes is known to have an all-around effect on the natural course of tuberculosis, including a higher risk of the individual becoming infected with *M. tuberculosis* (latent infection), a higher lifetime risk of tuberculosis activation, and an unfavourable clinical course. Presentation of more symptoms, more relapses, treatment failures and deaths [15; 16] were observed in tuberculosis combined with diabetes mellitus, especially those with uncontrolled diabetes [17]. Baseline bacterial load of tuberculosis with diabetes is higher than tuberculosis without, and the time to positive sputum conversion is prolonged [18]. It was reported that the 6 months percentage of positive sputum culture in tuberculosis patients with diabetes was 22.2%, compared with 6.9% in patients without diabetes [19]. According to a pooled analysis of four studies which adjusted for age and other potential confounders, diabetes was associated with nearly 5 times higher risk of death during tuberculosis treatment (pooled OR 4.95, 95%CI 2.69~9.10) [15] [20]. In a nationwide population-based study in an Asian population, a nearly 20% higher risk of 10 year accumulated all-cause mortality was observed in newly-diagnosed tuberculosis patients with diabetes than those without diabetes [21]. In addition, tuberculosis patients with diabetes showed atypical imaging changes and lesion distribution, especially in the lower lobe, while lesions in patients with tuberculosis only usually locate in the upper lobe. This has important clinical implications because tuberculosis occurring in the lower lobes is easily misdiagnosed as community-acquired pneumonia or tumors. Also, this has significant public health implications since early diagnosis and immediate initiation of treatment play a key role in tuberculosis control, especially in arresting tuberculosis transmission within a community.

The blueprint of WHO's End tuberculosis Strategy aims to reduce tuberculosis incidence by 80% and tuberculosis death by 90% by 2030 in the

world [22]. However, as mentioned previously, the effects of diabetes mellitus on the natural course of tuberculosis, may make the challenge for prevention and control of tuberculosis even greater. In the setting of the globally rapidly rising epidemic of type 2 diabetes, especially in low-to-middle income countries, the aggravation of the tuberculosis epidemic due to diabetes will pose a serious challenge to tuberculosis control for a relatively long period of time to come [23] [24]. In accordance, the WHO has recently identified T2D as neglected risk factor for the re-emergence of tuberculosis.

The Role of Vitamin D Deficiency in the Double Burden of Tuberculosis and Diabetes

In recent years, the non-skeletal function of vitamin D has aroused intense interest. Vitamin D is involved in the regulation of various genes in the body [25], and may be an important modulating factor in endocrine and immune functions. Vitamin D deficiency has been reported to be related to impaired insulin secretion and function, as well as anti-*Mycobacterium tuberculosis* immune response, and is significantly associated with the increased risk of diabetes and tuberculosis [26; 27; 28].

-The prevalence of Vitamin D deficiency in the Chinese population

Vitamin D deficiency is a global health problem [29] [30] [31]. High prevalence of vitamin D deficiency and insufficiency has been documented in Europe, China, India, Middle East and South America [30] [32], even in some areas with very sunny climates, such as Saudi Arabia and India [33] [34](Figure 1-2).
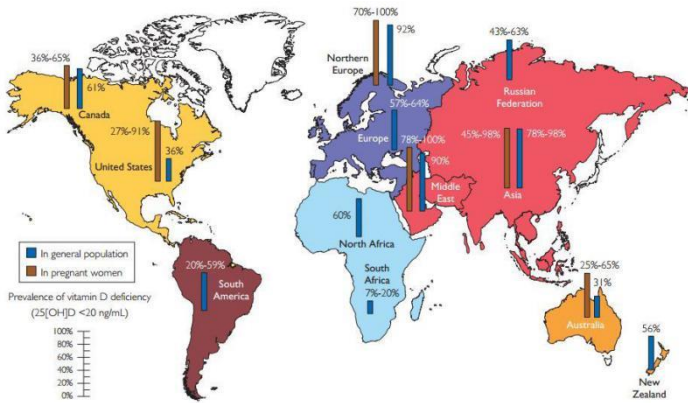
Figure 1-2 Reported incidence of vitamin D deficiency around the globe in pregnant women and the general population[35]

Some studies demonstrate that vitamin D deficiency is much higher in dark-pigmented populations and Asian populations due to a reduced ability of their skin to produce vitamin D [36]. Also, pregnant women, obese children and adults, and those who practice abstinence from direct sun exposure are at especially high risk [31] .

Sunlight exposure remains the major source of vitamin D. Vitamin D is mainly synthesized from 7-dehydrocholesterol in the skin under the action of ultraviolet light. Very few foods naturally contain vitamin D. These include oily fish such as salmon, mackerel and herring, mushrooms and cod liver oil. The absorbed or synthesized vitamin D3 undergoes hydroxylation in the liver and kidney to form 25-hydroxyvitamin D3 [25(OH)D$_3$] and 1,25-dihydroxycholecalciferol [1,25(OH)$_2$D$_3$]. 1,25(OH)$_2$D$_3$ is the main active form that performs the physiological functions of vitamin D in the body (Figure 1-3). 25(OH)D, the half-life of which is close to 3 weeks and the concentration of which in blood is stable, is the most valuable indicator for evaluating the individual nutritional status of vitamin D. The current widely accepted vitamin D nutritional status evaluation criteria are shown in Table 1-1 [37].
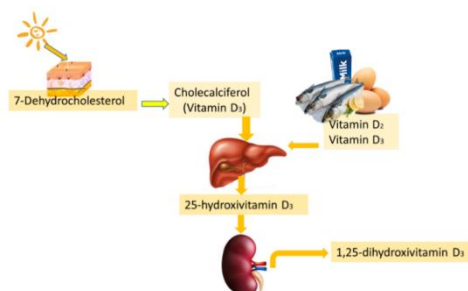
Figure1- 3 Main sources of vitamin D and its metabolism in the body

Table 1-1 The criteria of Vitamin D nutritional status in the body[37]

Serum 25(OH)D	Nutritional status
<10ng/ml(25nmol/L)	Severely deficient
<20ng/ml(50 nmol/L)	Deficient
<20 ~ 29ng/ml(52~72 nmol/L)	insufficient
≥30 ng/ml (75 nmol/L)	Sufficient
30 ~ 40 ng/ml(75-100nmol/ L)	Favorable
>150 ng/ml (374 nmol/ L)	Toxic

The prevalence of vitamin D deficiency in China is high [38] [39]. In 5531 (5-101 years old) urban Beijing residents reporting for health check-up, vitamin D deficiency and severe vitamin D deficiency percentages were 87.1 and 44.7, respectively [38]. A cross-sectional study including 6014 healthy elderly adults (≥ 60 years) showed that 34.1% of men and 44.0% of women presented with vitamin D deficiency [39]. A study of 418 Chinese immigrant population with mean age of 56 years in the Netherlands found that the prevalence of vitamin D deficiency in men and women was 67.9% and 53.1%, respectively [40]. Although there are some discrepancies in the reported vitamin D

deficiency rates due to different season of blood collection, analysis method, etc, vitamin D deficiency certainly is a great public health problem in the Chinese population.

The public health consequences of vitamin D deficiency are profound. In recent years, there has been an increasing number of studies focusing on exploring the relationship between vitamin D deficiency and metabolic abnormalities, such as hypertension, dyslipidemia, central obesity, glucose intolerance, type 2 Diabetes, as well as infectious diseases like tuberculosis.

- *Vitamin D Deficiency and Diabetes Mellitus*

Epidemiological evidence for an inverse association between serum vitamin D levels and the risk of type 2 diabetes is abundant. A cross sectional study in overweight and pre-diabetic populations in China (2813 males with mean age 52·7 years, 3784 females with mean age 52·3 years) reported 83·3 % vitamin D deficiency, and vitamin D status was inversely associated with HOMA-IR (homeostasis model assessment of insulin resistance) [41]. Cohort studies provided stronger proof. A recent study in Asia found that compared to individuals with vitamin D concentrations ≥ 20 ng/mL, those with concentrations of 10-19.9 ng/mL and of <10 ng/mL have a 2.06 and 3.23 fold increased risk of developing type 2 diabetes, respectively [28].

The effects of vitamin D supplementation on insulin resistance and glycemic control give more insight in the correlation between vitamin D and risk of diabetes. In non- diabetic adults with impaired fasting glucose at baseline, calcium and vitamin D supplementation for 3 years attenuated the increases in glycemia and insulin resistance [42]. Improved insulin secretion was reported for vitamin D supplementation in women with type 2 diabetes [43]. However, some other studies did not find effects of vitamin D supplementation on glucose metabolism [44 ; 45]. Vitamin D supplementation of a bolus oral dose of 100,000 IU cholecalciferol followed by 4000 IU cholecalciferol/d for 16 weeks did not improve insulin sensitivity or secretion in overweight or obese adults with vitamin D deficiency [46].

In addition, low serum vitamin D level has been observed to be associated with impaired glycemic control in diabetes [47] . And a RCT study found a significant effect of vitamin D supplementation on HbA_{1c} after 6 months supplementation in severe vitamin D deficient patients [48].

Summarizing, there is convincing evidence that vitamin D deficiency is associated with higher risk of diabetes, as well as with impaired glycemic control in diabetes patients.

- *Vitamin D Deficiency and Tuberculosis*

Previous studies have suggested that low serum 25(OH)D may be associated with acquiring wheezing and lower respiratory tract infections and with the disease severity both in children [49] and in adults [50], indicating the possible link between vitamin D deficiency and increased susceptibility to infection. A meta-analysis in 2008 combining 7 epidemiological studies, showed a modest to strong association of vitamin D deficiency with tuberculosis risk, with a pooled effect size of 0.68 (95% CI, 0.43~0.93), i.e. serum vitamin D levels are 0.68 SD lower in people with tuberculosis compared to controls [51]. In a global country-based ecological study, across 154 countries, annual solar UV-B exposure was associated with tuberculosis incidence (averaged over the period 2004-2013). Tuberculosis incidence in countries in the highest quartile of UV-B exposure was 78% lower than that in countries in the lowest quartile, and 6.3% global variation in tuberculosis incidence could be attributed to variations in annual UV-B exposure [52]. Further, several prospective longitudinal studies in recent years discovered the association between vitamin D deficiency and possible higher risk of active tuberculosis [53; 54; 55]. Higher serum vitamin D concentration was associated with low incidence of tuberculosis infection conversion (TBIC) (P trend=0·005), and an increase of 1ng/mL vitamin D concentration decreased the incidence of TBIC by 6% (relative risk 0.94, 95% CI 0.90-0.99, P=0.015) [53]. Low 25-hydroxyvitamin D (<32ng/mL) in South African infants was associated with

higher risk of tuberculosis (adjusted hazard ratio 1.76, 95% CI 1.01–3.05; p = 0.046), as well as significant interaction with two SNPs (Single nucleotide polymorphisms) that are considered to be associated with innate immunity [54].

In fact, vitamin D3 isolated from cod liver oil was used for tuberculosis treatment in the 1930s and widely introduced in the pre-antibiotic era. Starting in the 1950s, anti-infective chemotherapy was introduced, and vitamin D was no longer used for clinical treatment. At present, there is still controversy about the efficacy of adjunctive use of vitamin D on tuberculosis treatment. Some studies reported that adjunct therapy of vitamin D3 to standard therapy may have beneficial effects towards clinical recovery. This includes sputum culture conversion [56], weight gain and chest radiographic findings [57], resolution of inflammatory responses that are associated with increased risk of mortality [58]. Furthermore adjunct therapy may modulate specific cytokine levels that mediate immune cell to cell signaling such as IL-1β signaling. This is critical for signaling between macrophages and lung epithelial cells, leading to epithelial antimicrobial peptide production that helps to contain *Mtb* infection [59]. However, other trials did not find a significant effect of vitamin D concomitant with standard first-line anti-tuberculosis drugs on improving the rate of sputum *Mtb* clearance [60; 61; 62]. A recent study reported that the improvement of sputum clearance by high dose vitamin D (four biweekly doses of 140,000 IU vitamin D_3) was modified by SNPs in genes encoding the vitamin D receptor (VDR) and 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) [63].

Recent meta-analyses reinforce the finding that 10-20 µg per day of vitamin D can reduce all-cause mortality and cancer mortality in middle-aged and older people. However, a role of vitamin D to improve non-skeletal health conditions including glucose metabolism and tuberculosis has not been confirmed with sound evidence in randomized trials till now [64]. Therefore, clinical studies are urgently needed to verify the possible adjunctive role of vitamin D. Adjunctive use of vitamin D in active tuberculosis combined with diabetes merits further investigation.

In summary, diabetes triples the risk of tuberculosis. The rapidly increasing diabetes incidence and still high burden of tuberculosis in China poses a new challenge for public health. Therefore, the prevention and control of tuberculosis may still be a significant task in the coming decade. Vitamin D deficiency is associated with increased risk of diabetes mellitus as well as the severity of the disease. At the same time, vitamin D deficiency is correlated with increased risk of tuberculosis independently of diabetes. We hypothesize that the double burden of diabetes and tuberculosis and its implications to public health in this country are heavily influenced by the high prevalence of vitamin D deficiency (**Figure 1-4**).
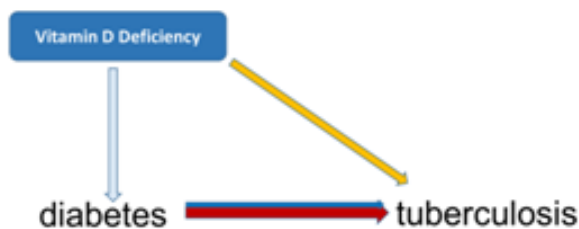
Figure 1-4 Working hypothesis of a double burden of diabetes and tuberculosis and the role of vitamin D deficiency

The red arrow represents the effect of diabetes mellitus on tuberculosis risk; The yellow arrow represents an independent effect of vitamin D deficiency on tuberculosis risk; The light blue arrow represents an effect of vitamin D deficiency on diabetes mellitus risk and severity; the dark blue arrow represents an additional effect of vitamin D deficiency on tuberculosis risk mediated by diabetes.

Possible Mechanisms Underlying the Double Burden of Diabetes and Tuberculosis

Although it is widely accepted that diabetes weakens the immune system through impairing both innate and adaptive immune functions, and that vitamin D is also involved, the biological basis underlying the correlation between diabetes and tuberculosis is poorly understood.

-impaired immune responses to M. tuberculosis due to diabetes

Dysregulation of the immune system was observed in diabetic patients, involving innate and adaptive immune responses, especially with respect to macrophage and T lymphocyte function that play a key role in the defence against M. tuberculosis [65] [66] [67; 68] [69]. This was especially the case in those with chronic hyperglycemia [70]. A significant decrease in the activity of natural killer cells was found in diabetic patients, making them more susceptible to infection with respiratory pathogens such as M. tuberculosis [71]. Macrophages are key to the progression of tuberculosis due to their dual role, forming a primary host cell reservoir for M. tuberculosis, as well as being effective cells that control and eliminate M. tuberculosis [72]. Recent evidence shows that human monocytes and macrophages were altered in the context of T2D [73; 74] , resulting in impaired anti- *Mycobacterium tuberculosis* immunity.
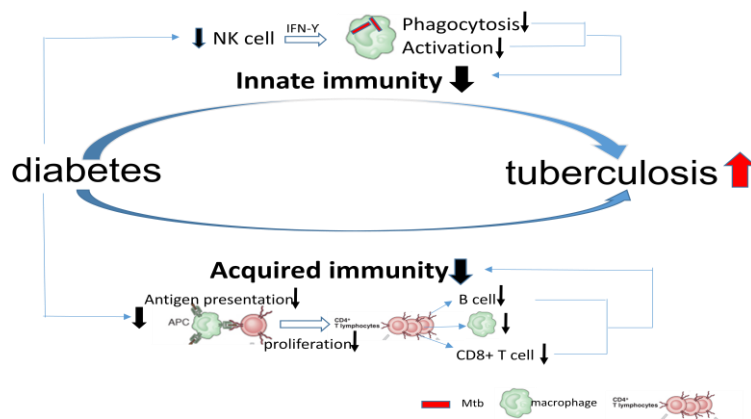
Figure 1-5 The possible immunity related mechanism of the increased risk of tuberculosis in diabetes

It was reported that tuberculosis patients with diabetes had a different profile of circulating levels of cytokines compared with those without diabetes [75], which indicates that cytokine profile may be a biomarker of the immunity response to M.tuberculosis in diabetes patients. Cytokines play an important role as mediator in the interaction between immune cells, notably IFN-Y and TNF-α, the functions of which have been well documented [76] [77]. This interaction results in macrophage activation to control mycobacterial replication, and the production of granulomas which contain the bacterium. Type 1, type 17, and the IL-1 family of cytokines have been implicated in protection against M. tuberculosis whereas type 2 and anti-inflammatory cytokines such as IL-10 frequently are associated with either increased susceptibility to disease and/or enhanced pathology [78]. Leptin, with a main role in regulating body weight, is also involved in both innate and adaptive immunity [79] [80] [81]. Leptin receptors have been found in neutrophils, monocytes, and lymphocytes. Systemic levels of leptin were reported to be lowered in tuberculosis patients with diabetes compared to tuberculosis without diabetes, indicating a potential contribution to the pathogenesis of tuberculosis in diabetes [82; 83]. A recent meta-analysis of

twelve case-control studies found serum leptin levels of healthy controls were markedly higher than those of tuberculosis patients [84], and an increase in plasma leptin levels after treatment of tuberculosis was reported [85].

The possible immunity mechanism of the increased risk of tuberculosis in diabetes is shown in figure 1-5.

- possible mechanism of Vitamin D deficiency underlying the combination of diabetes and tuberculosis

The vitamin D receptor (VDR) can be demonstrated in many organs and this suggests that vitamin D metabolites may have many extra-skeletal effects. The VDR is present in the pancreatic β-cell, activated T Cells, B cells, macrophages, monocytes, etc.

Vitamin D has important regulatory effects on insulin secretion and insulin signaling. vitamin D binds to its receptor to activate L-type calcium channels on beta cells, promoting insulin release and tyrosine phosphorylation of insulin receptor substrates, and initiating insulin signaling. Lack of vitamin D may cause the calcium channel to shut down, or the phosphorylation of the insulin receptor substrate to be blocked, thereby affecting insulin signaling.

In recent years, the immunomodulatory effects of vitamin D both in the innate and adaptive immune system have been widely accepted. Significant concentrations of VDR have been found in immune cells which have important effects in bactericidal activities in the body. Vitamin D was discovered to stimulate innate immunity during M. tuberculosis infection resulting in control of *M.* tuberculosis proliferation inside macrophages [86]. The active metabolite of vitamin D, 1, 25-dihydoxyvitamin D, has long been known to enhance the immune response to mycobacteria in vitro. When vitamin D binds to the macrophage membrane Toll-like receptor, it activates 1-alpha hydroxylase and induces the expression of the anti-microbial peptide Cathelicidin. In turn, this inhibits and kills intracellular M.

tuberculosis [87]. In tuberculosis patients with vitamin D deficiency, this polypeptide was reduced in granulomatous lesions [88]. Similarly, Wang et al. found that vitamin D regulates the expression of polypeptide β-defensin on the mucosal surface [89], which is also an antimicrobial polypeptide with multiple effects in the immune system. Vitamin D was reported to be involved in the regulation of host cytotoxic T lymphocyte responses and the differentiation of naive T cells to regulatory T cells, rather than to T helper type 1(Th1) or Th17 cells [90], indicating its possible role in adaptive immunity during infections (Figure 1-6).

A recent report proposed that vitamin D deficiency in diabetes patients may increase tuberculosis susceptibility, based on the observation that the intracellular mycobacterial growth in monocytes obtained from T2D patients with vitamin D deficiency was significantly higher than in healthy volunteers, while no difference existed between T2D patients without vitamin D deficiency and healthy volunteers [91].

Tregs: regulatory T cells
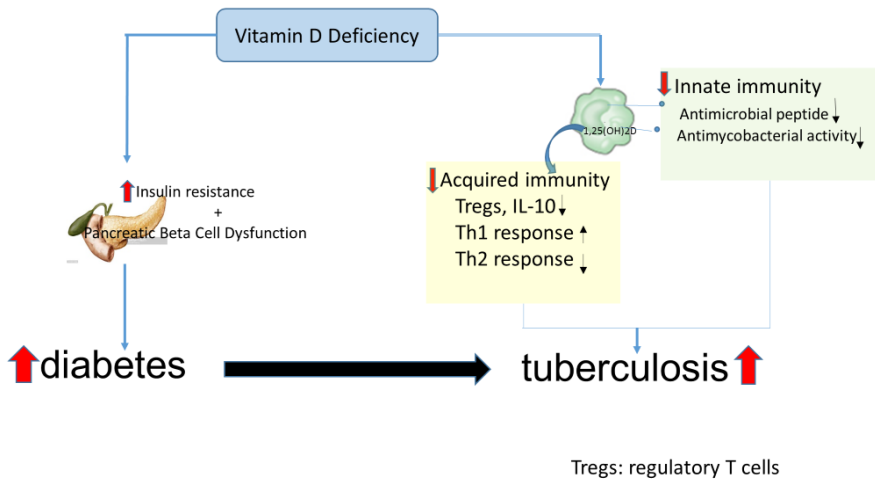
Figure 1-6 **Mechanism of vitamin D deficiency on diabetes and tuberculosis**

Research objectives

The worldwide trend of increasing obesity, diabetes and still high prevalence of tuberculosis in low to middle-income areas constitute a double burden which will challenge public health systems in the coming decades. Multidrug resistant tuberculosis will complicate the situation even more. Also, the endemic vitamin D deficiency may play a role, due to its association with both immune function and insulin secretion and resistance. If so, supplementation may open perspectives for prevention and even therapy. Therefore, we carried out this epidemiologic study with the purpose:

1. To investigate the epidemiology of concurrent tuberculosis with diabetes.
2. To investigate the risk factors of co-occurrence of tuberculosis and diabetes.
3. To identify the role of vitamin D in the risk of diabetes and tuberculosis, and their co-occurrence.
4. To explore whether adding vitamin D to standard therapy can improve the prognosis of tuberculosis with and without diabetes mellitus.

Outline of the thesis

First, a large epidemiological survey was carried out among newly diagnosed tuberculosis patients and non-tuberculosis controls from a rural community, to screen for diabetes (Figure 1-7). In addition, we explored possible risk factors for concurrence of the diseases, in order to provide a scientific basis for early diagnosis of tuberculosis in diabetes. To our knowledge, this is the first large scale survey of diabetes mellitus in tuberculosis patients in the community **(chapter 2)**.
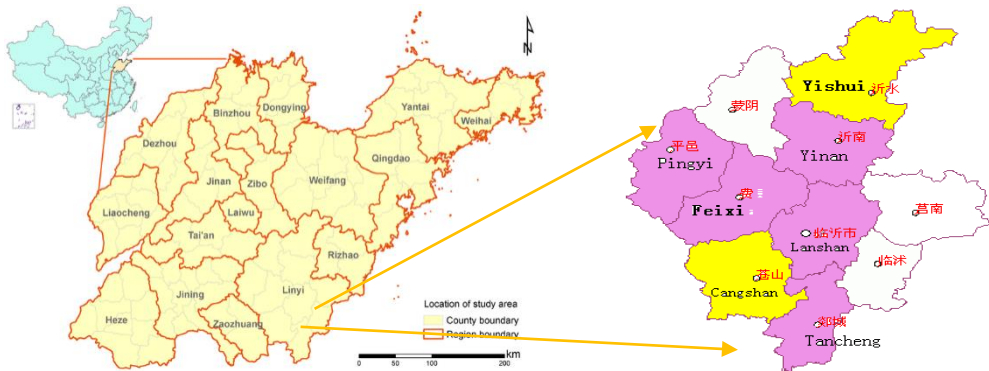
Figure 1-7 The study area of Linyi, Shandong

Many tuberculosis patients have a significant vitamin D deficiency, and this may be closely related to the onset and progress of the disease. To investigate whether diabetes may influence the time lag from onset of typical symptoms until diagnosis of tuberculosis, we randomly selected patients from the epidemiological survey in chapter 2 (**Chapter 3**). Four hundred and sixty-one active tuberculosis patients (192 with diabetes and 269 without diabetes) were randomly selected from a city level chest hospital (latitude 36° N) to evaluate the vitamin D status and the possible predictors of vitamin D deficiency. And we investigated whether vitamin D may be a possible link between tuberculosis and diabetes in (**chapter 4 and chapter 5**). In a subsequent study, cellular immune function related cytokine levels in tuberculosis patients with and without diabetes were observed, with further analysis of their relationship with serum 25(OH)D (**Chapter 6**).

A cluster randomized trial of vitamin D supplementation added to pharmacotherapy in tuberculosis patients with and without diabetes was carried out in a city-level chest hospital. Possible effects of vitamin D supplementation on treatment outcomes were observed. The main purpose was to further explore the role of vitamin D in tuberculosis and diabetes (**Chapter 7**).

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2

Prevalence of Type 2 Diabetes among Newly Detected Pulmonary Tuberculosis Patients in China: A Community Based Cohort Study

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Abstract

Background: Patients with type 2 diabetes (DM) have a higher risk of developing pulmonary tuberculosis (PTB); moreover, DM co-morbidity in PTB is associated with poor PTB treatment outcomes. Community based prevalence data on DM and prediabetes (pre-DM) among TB patients is lacking, particularly from the developing world. Therefore we conducted a prospective study to investigate the prevalence of DM and pre-DM and

evaluated the risk factors for the presence of DM among newly detected PTB patients in rural areas of China.

Methods and Findings: In a prospective community based study carried out from 2010 to 2012, a representative sample of 6382 newly detected PTB patients from 7 TB clinics in Linyi were tested for DM. A population of 6674 non-TB controls from the same community was similarly tested as well. The prevalence of DM in TB patients (6.3%) was higher than that in non-TB controls (4.7%, p<0.05). PTB patients had a higherodds of DM than non-TB controls (adjusted OR 3.17, 95% CI 1.14-8.84). The prevalence of DM increased with age and was significantly higher in TB patients in the age categories above 30 years (p<0.05). Among TB patients, those with normal weight (BMI 18.5-23.9) had the lowest prevalence of DM (5.8%). Increasingage, family history of DM, positive sputum smear, cavity on chest X-ray and higher yearly income (≥10000 RMB yuan) were positively associated and frequent outdoor activity was negatively associated with DM in PTB patients.

Conclusions: The prevalence of DM in PTB patients was higher than in non-TB controls with a 3 fold higher adjusted odds ratio of having DM. Given the increasing DM prevalence and still high burden of TB in China, this association may represent a new public health challenge concerning the prevention and treatment of both diseases.

Introduction

The association between diabetes mellitus (DM) and tuberculosis (TB) has been recognised for centuries. DM was a well-known risk factor for TB[1] , but this association was nearly forgotten after the advent of widely available

treatment for both diseases. With the current global increase in DM largely driven by increasing prevalence in the developing world, the DM population is anticipated to reach 552 million by 2030[2] and the link is re-emerging. The co-morbidity of DM and TB represents a double burden with significant public health implications as recently recognised by several authors[3][4] . In developing countries such as India, China, Bangladesh, Indonesia and Brazil, where TB is still highly endemic[5], the double burden and interaction of DM and TB will be more ominous. China accounts for nearly 17% of the world's TB burden, with an estimated 1.5 million new cases and approximately 270,000 deaths each year. In the meantime, the country has also witnessed an escalating epidemic of DM[2] as the consequence of industrialization, urbanization, increase in life expectancy, and changes in lifestyle in recent years. It was recently reported that the agestandardised prevalence of DM and impaired glucose tolerance (IGT) reached to 9.7% and 15.5% respectively in China[6].

Jeon CY et al carried out a large meta analysis and discovered that DM patients were 3.1 times (95% CI 2.27 - 4.26) more likely to have TB than non-diabetic controls[7]and it has been estimated that the TB risk attributable to DM was between 15% and 25%[8][9]. Two large scale longitudinal cohort studies from Korea and UK have shown similar findings with risk ratios of 3.47 (95% CI 2.98 - 4.03) and 3.80 (95% CI 2.30 - 6.10)[10][11]. The mechanism behind the association between TB and DM is not fully understood but studies suggest that DM depresses the immune response through effects on macrophage and lymphocyte function, which in turn facilitates active TB disease. Conversely, it is also possible that TB can induce glucose intolerance and also deteriorate glycemic control in subjects with DM [12].

However, only a few studies have reported on screening for DM in TB patients. A wide range of DM prevalence among TB patients (1.9% to 39%)

has been reported and most of the studies are based on secondary data analysis, self-reported DM, or have a small sample size[7][10][13]. Gaining a deeper understanding of the differences between TB patients with and without DM is urgently needed to prevent the co-morbidity and to improve the prognosis of the patients with DM and TB. Therefore, we initiated this large scale prospective epidemiologic study using primary data to identify the current prevalence of DM and pre-DM in newly- diagnosed PTB patients together with non-TB controls from the same community. Also, the odds ratios for DM among TB patients were analysed in order to get the clues to the early detection of DM in TB patients, and to figure out DM patient with what characteristics should be given priority to the prevention of TB.

Methods

Ethics Approval

This study was carried out in accordance with requirements documented in the Declaration of Helsinki. Ethics approval was obtained from the medical ethics committee of Qingdao Disease Prevention and Control Centre, Qingdao, People's Republic of China. All participants were fully informed and gave their written informed consent. This trial is registered in the Chinese Clinical Trial Registry (No. ChiCTR – OCC - 10000994, URL: http://www.chictr.org/cn/proj/show.aspx?proj = 411).

Study Population

The study population was selected from Linyi rural area, Shandong province in North China. Seven TB clinics were randomly selected for this study including Yishui, Yinan, Lanshan, Cangshan, Tancheng, Feixian and Pingyi. Each TB clinic had a defined catchment area comprising approximately 0.9

million inhabitants. The diagnosis of PTB was made within the existing TB prevention and control system in China, in which clinical manifestations, sputum smear microscopy and chest radiography were the central component. Suspected PTB person was investigated by sputum smear examination. The patient was diagnosed as smear-positive PTB if sputum specimens were smear positive; if sputum smears were negative and chest radiograph was compatible with active PTB, the patient was diagnosed as smear-negative PTB after discussion by clinical and radiographic doctors[9]. All adult (≥18 years) newly-diagnosed PTB patients who registered for Directly Observed Treatment, Short Course (DOTS) in these TB clinics from September 2010 to December 2012 were included. HIV-positive patients were excluded because of the influence of antiretroviral therapy on insulin resistance as were subjects with type 1 diabetes.

A sample size of 7000 was calculated, assuming a prevalence of DM as 6.7% amongst the TB subjects as reported in the literature[14], considering non-response rate of 20%[15]. We adjusted it to 6200 because of better compliance of the participants observed in the pilot study than expected (modifying non-response rate to 10%). For estimating DM and pre-DM prevalence amongst the non-TB cohort, cluster random sampling was used to recruit subjects from the same communities as the TB cases.

Diagnosis of DM and pre-DM was based on WHO criteria for the classification of glucose tolerance based on fasting plasma glucose (FPG)[16]. After an overnight fast, venous blood of each participant was collected. Glucose oxidase method was used to estimate FPG level. Those with FPG≥6.1 mmol/L were referred to DM clinics for diagnostic confirmation with a second FPG test. Those with FPG level in the range of 6.1 mmol/L to 6.9 mmol/L were screened as pre-DM; those with FPG level ≥7.0 mmol/L were screened as DM. Lipid indexes including total cholesterol, triglyceride

and HDLC were estimated by enzymatic procedure. Anthropometric measurements including height and weight by standard procedure were measured by trained investigators. Body mass index (BMI, kg/m²) was calculated by using the formula: BMI = Weight (kg)/Height² (m²). Underweight, normal weight, overweight, and obesity were defined by using the modified criteria for Chinese population [17]. The BMI cut-off value for underweight (severe underweight, moderate underweight, mild underweight), normal weight, overweight, and obesity was < 18.5 kg/m2 (< 16 kg/m², 16- 16.9 kg/m², 17-18.4 kg/m²), 18.5-23.9, 24.0-27.9 kg/m² and ≥28.0 kg/m²,respectively. Two blood pressure measurements were taken using sphygmomanometer with the subject in sitting posture, and the average of the two readings was recorded.

A structured questionnaire was administered to obtain information regarding socio-demographics, personal and family disease history, and lifestyle risk factors including smoking, alcohol consumption, educational level, outdoor activity, yearly income and marital status. The interviews were conducted by trained local TB workers in order to assure compliance and quality of the data collected.

Data Management and Quality Control

Double data entry was carried out, and then a computer based error detection system was used to check the consistency of data. In case of inconsistency, the original questionnaire was checked and the error corrected via a re-entry. During the investigation, different methods of quality control including inter-lab comparison of glucose measurement were carried out.

Statistical Analysis

The study design followed STROBE Guidelines[18]. SPSS version 19.0 was used for statistical analysis. Characteristics of TB and non-TB controls were compared and the study characteristic of TB patients by diabetes status including normoglycemia (Non-DM), prediabetes (Pre-DM), and diabetes (DM) were analyzed. Mean and standard deviation for continuous variables and proportions for categorical variables are reported. Independent sample t test and ANOVA were used to test continuous variables. Chi-square or Fisher's exact test was used to compare categorical variables. Kruskal-Wallis H test was used to compare ranked variable. Two multinomial logistic regression analyses were performed. The variables for inclusion in the multivariate model were chosen based on plausibility and variables with p values of < 0.1 in univariate analysis were entered into the multivariate analysis. One multivariate logistic analysis was to examine the association between PTB and DM and pre-DM and to calculate odd ratios and 95% confidence intervals. The dependent variable was either DM or pre-DM, the independent variables being suffering from active PTB and we examined the following covariates for the effect modification or confounding: age [(years) (categorized in 4 units: < 30, 30 - 39, 40 - 49, ≥50)], sex, BMI [(kg/m²) (categorized as < 18.5, 18.5 - 23.9, ≥24.0)], yearly income[(RMByuan) (categorized as < 2000, 2000 - 9999, ≥10000)], family history of DM, smoking, alcohol consumption, outdoor activity, education level and marital status. The other logistic regression analysis was performed to qualify the odds of having DM and pre-DM in active PTB patients, the dependent variable being either DM or pre-DM and the independent variables being the same as the first model without active TB and with PTB profile including positive sputum smear, cavity and involved lung field on chest radiograph. The entry probability was p≤0.05 and the removal probability was p>0.10. All variables were checked for collinearity in both models. The model fit was

significant for both multinomial logistic regression analyses (x^2 = 285.6 and 372.8, respectively, p<0.001) and the fit was good (x^2 = 1093.47 and 1067.52, respectively, p>0.05). A p value of <0.05 was considered statistically significant.

Results

The details on the total number of eligible PTB patients, those who gave written consent to undergo screening of DM and those included in the final analysis are illustrated in a flow chart (Figure 2-1). A total of 8410 patients of ≥18 years of age were registered for TB treatment in the above 7 TB clinics in the study period. Finally, 6902PTB patients completed the study. After the exclusion of 520 persons for whom demographic information or fasting glucose levels were missing, 6382 patients (4627 men and 1715 women) were included in the final analysis. The recorded response rate was 92.5%.
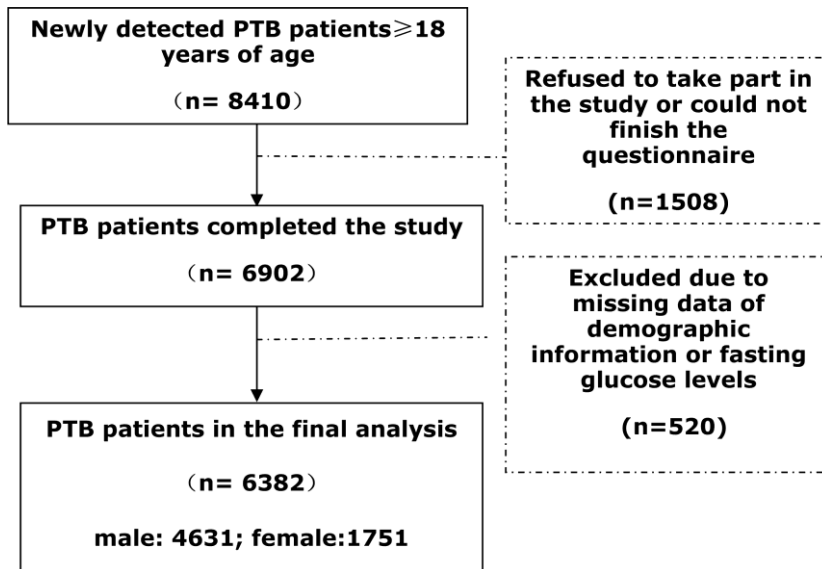
Figure 2-1. Flowchart of the pulmonary tuberculosis patients in the study, Linyi, 2010-2012.

The details on the total number of eligible participants, those who have given written consent to undergo screening of DM and the patients included in the final analysis are illustrated in Figure 1. There were 8410 newly detected pulmonary tuberculosis patients in total study period. Finally, 6382 patients were included in the final analysis.

General Characteristics

The demographic and anthropometric information of the TB and non-TB cohort is detailed in Table 2-1.

Data from 6382 TB patients and 6675 non-TB controls was available for analysis. The mean age of the PTB patients was 50.4±18.6 and of the non-TB controls 50.8±16.4 (p>0.05). There was a significant sex difference between TB and non-TB (p<0.001). Mean BMI of non-TB cohort was significantly higher than that of the TB cohort (p<0.001). Systolic blood pressure and diastolic blood pressure were higher in non-TB cohort compared to TB cohort (p<0.001). Plasma glucose level was higher in TB patients compared

to non-TB controls (p<0.001). Smoking was more common in TB patients than in non-TB controls (p<0.001), while alcohol consumption was higher in non-TB (p<0.001).

Table 2-1.   Characteristics of TB patients and non-TB controls, Linyi, 2010–2012.

	TB(n=6382)	**Non-TB**(n=6675)	**p value**
Male, n(%)	4631(72.90)	3712(55.61)	<0.001
Age	50.41±18.63	50.77±16.40	0.242
BMI	20.91±2.76	22.52±2.99	<0.001
Sbp	119.69±11.43	123.95±15.00	<0.001
Dbp	76.70±7.70	79.31±9.80	<0.001
Plasma glucose	5.39±1.88	5.15±1.20	<0.001
Hemoglobin	131.89±17.25	131.07±43.48	0.610
Total cholesterol	4.39±1.09	4.69±1.20	<0.001
HDLC	1.46±0.60	1.63±0.82	<0.001
Triglyceride	1.20±1.11	1.31±0.94	0.884
DM Family history, n (%)	695(11.4)	765(12.6)	<0.001
Smoking, n (%)	900(14.1)	379(5.7)	<0.001
Alcohol consumption, n (%)	414(6.5)	753 (11.6)	<0.001
Yearly income, n(%)			
<2000	883(13.9)	935(14.0)	0.002
2000~9999	3937(61.9)	4507(67.5)	
≥10000	1541(24.2)	1232(18.5)	
Marital status, n(%)			
Married	4694(79.4)	5419(87.8)	<0.001
Single/widowed/divorced	1221(20.6)	754(12.2)	
Educational status, n(%)			
illiteracy	1625(27.4)	1553(25.2)	<0.001
primary or middle school	3558(59.9)	3300(53.5)	

high school or higher	758(12.8)	1312(21.3)	
Outdoor activity, n (%)			
>2 hours/d	3779(63.6)	4041(67.5)	<0.001
≤2 hours/d	1863(31.4)	1644(27.5)	
None	300(5.0)	302(5.0)	

Relationship Between PTB and DM

Details of the DM and pre-DM prevalence in TB patients and non-TB controls are shown in Figure 2. Based on FPG, out of 6382 TB patients, 403 (6.3%) had DM; while out of 6675 non-TB controls, 313 (4.7%) had DM. The prevalence of DM in TB patients was significantly higher than in non-TB controls (p<0.05). In males, the prevalence of DM was 52% higher in TB than in non-TB (p<0.05). Taking into account possible confounding factors such as age, sex, BMI, family history of DM, education level, smoking, alcohol consumption, outdoor activity and marital status, we found that PTB patients had 3.17 times higher odds of having DM compared with non-TB control (Table 2-2).

Table2-2 Odds Ratio for diabetes and pre-diabetes by PTB in Linyi, China, 2010–2012.

	Diabetes			
	Crude OR	**P**	**Adjusted OR[*]**	**P**
overall	1.38(1.19-1.61)	<0.001	3.17(1.14-8.84)	0.027
male	1.59(1.35-1.94)	<0.001	3.27(1.06-10.06)	0.039
	Pre-diabetes			
	Crude OR	**P**	**Adjusted OR[*]**	**P**
overall	1.15(1.01-1.32)	0.041	1.14(0.50-2.57)	0.76
male	1.25(1.05-1.48)	0.013	1.09(0.46-2.56)	0.84

[*]Adjusted for age, BMI, sex, family history of DM, yearly income, education level, smoking, alcohol consumption, outdoor activity, marital status.

The prevalence of DM increased with increasing age and was significantly higher in the TB groups than in the non-TB groups except for the age subgroup <30. The same tendency existed for pre-DM but the difference was only significant in the age subgroup of 30 - 39 (Figure 2-2).

Among BMI subgroups in TB patients, those with normal BMI (18.5 - 23.9) had the lowest prevalence of DM (5.8%), while the prevalence of DM in overweight and obese cases (BMI≥24) was the highest (7.7%). The greatest difference (2.6 fold) in DM prevalence between TB and non-TB was observed in the underweight group (p<0.05) (Figure 2-2).

DM was newly detected in 177 (43.9%) PTB patients and in 136 (43.5%) non-TB controls, respectively. In general, patients with previously diagnosed DM had higher fasting plasma glucose levels than did those with newly-diagnosed DM. 10.3% of previously known DM cases had their blood glucose level tested regularly. The prevalence of pre-DM in TB patients and non-TB controls was 7.4% and 6.6%, respectively, all previously undiagnosed.
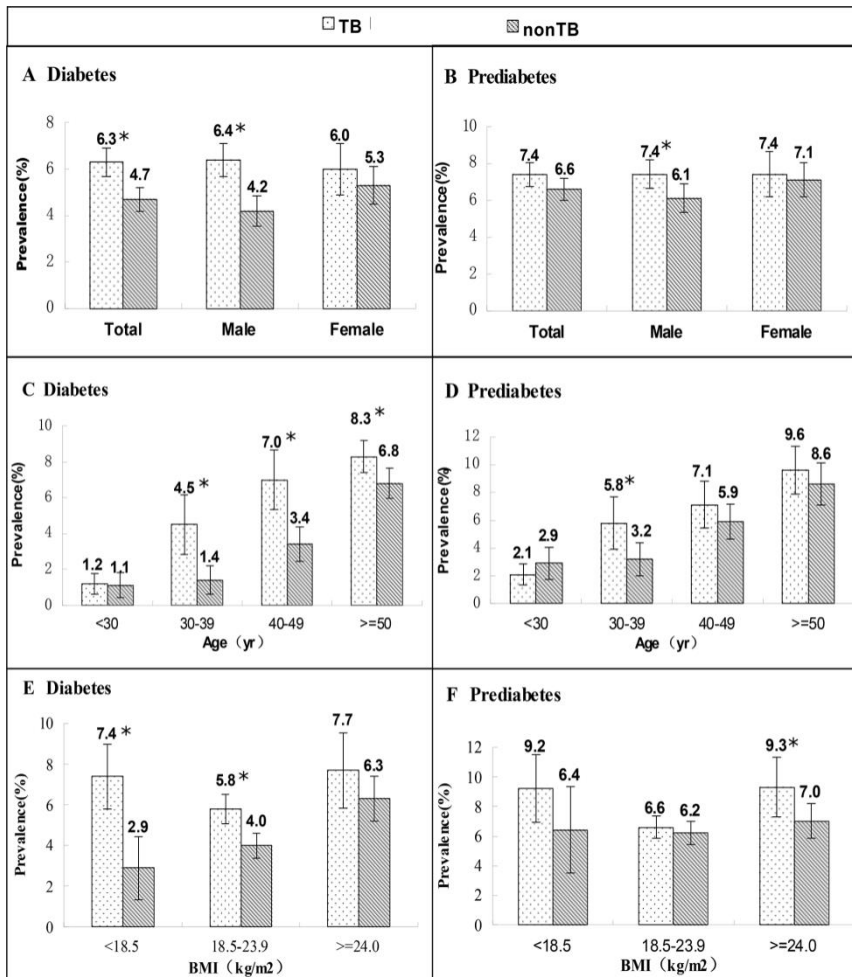
Figure 2-2. The prevalence of diabetes and prediabetes in TB and non-TB.

The overall prevalence of diabetes and prediabetes in TB and non- TB was shown in Figure 2. Also, the sex, age (<30, 30<39, 40 - 49, ≥50) and BMI (<18.5, 18.5 - 23.9, ≥24.0) stratified prevalences were shown. The prevalence of diabetes in PTB patients was 6.3%, which was significantly higher than in non-TB controls (4.7%). The prevalence of diabetes increased with increasing age and was significantly higher in the TB than in the non-TB groups except for the age group <30. Among BMI subgroups in TB patients, normal weight patients with a body mass index of 18.5 - 23.9 had the lowest prevalence of DM (5.8%), while the prevalence in overweight and obese cases (BMI≥24) was the highest. TB: pulmonary tuberculosis patients, non-TB: non tuberculosis controls.

Characteristics of TB Patients with DM or Pre-DM

Table 2-3 shows the comparison of the study characteristics of PTB patients with Non-DM, Pre-DM, and DM.

Among PTB cases (6382), 403 patients had DM and 470 had pre-DM. PTBpatients with DM and pre-DM were older than the subjects with normoglycemia (57.81, 57.11 vs 49.29, p<0.001). PTB patients with DM had a higher systolic and diastolic blood pressure in comparison with patients with normoglycemia and pre-DM (p = 0.024, 0.017, respectively). Triglyceride and total cholesterol were higher compared with patients with normoglycemia (p = 0.024, <0.001, respectively).

Profile of PTB also showed significant difference. A higher proportion of PTB patients with DM were smear positive (61.6%) compared to those with normoglycaemia (46.8%). A small proportion of patients were categorised as PTB relapse, and patients with DM had the highest relapse rate (p<0.001).

Table 2-3 Study characteristics of TB patients by diabetes status in Linyi, China, 2009-2012

Characteristics	Non-DM	Pre-DM	DM	P value
N	5509	470	403	
Male, n(%)	3992(72,5)	341(72.6)	298(73.9)	0.813
Age(years)	49.29±18.97	57.11±14.72[*]	57.81±14.23[*]	<0.001
BMI(kg/m^2)	20.90±2.72	20.86±2.97	21.09±3.06	0.234
Underweight	866(17.2)	95(21.8)[*]	77(20.8)[*]	<0.001
Normal	3504(69.5)	265(60.8)[*]	231(62.3)[*]	
Overweight and obesity	674 (13.4)	76(17.4)[*]	63(17.0)[*]	
Blood Pressure(mmHg)				
Systolic	119.53±11.26	119.91±11.77	121.62±13.07[*]	0.024
Diastolic	77.51±7.70	77.85±6.78	78.61±8.61[*]	0.017

Fasting glucose(mmol/l)	4.94±0.60	6.44±0.26*	10.30±4.81*&	<0.001
Haemoglobin(g/L)	131.88±16.90	132.30±18.99	131.54±20.08	0.762
Triglyceride(mmol/l)	1.19±1.09	1.23±1.47	1.35±0.74*	0.024
Total cholesterol(mmol/l)	4.34±1.01	4.61±1.24*	4.75±1.62*	<0.001
HDLC(mmol/l)	1.44±0.59	1.65±0.74*	1.47±0.52&	<0.001
Type of PTB*, n(%)				
Smear positive cases	2513(46.8)	237(55.3)	198(61.6)	<0.001
Smear negative cases	2857 (53.2)	192(44.7)	123(38.4)	
Relapse, n(%)	247 (4.6)	24(5.6)	28(8.8)	<0.001

*P<0.05 compared with non-DM group, & P<0.05 compaired with pre-DM group

Odds Ratios for DM and Pre-DM among PTB Patients by Multivariate Risk Assessment

In the multivariate logistic regression models, increasing age, family history of DM, positive sputum smear, cavity on chest X- ray and higher yearly income (≥10000RMB yuan) were significantly associated with an increased odds of DM in TB patients. Underweight had a borderline significant association with DM compared with normal weight (OR = 1.30, p = 0.082). Frequent outdoor activity was negatively associated with DM odds. Age category of ≥50 years had the highest odds ratio of 13.20 (p<0.001), followed by age category of 40 - years and family history of DM with OR 9.46, 5.85 respectively. Associations were not observed for sex, education, smoking, alcohol consumption, marital status and involved lung field on chest X-ray (Table 2-4).

As to having pre-DM, age, family history of DM, overweight, obesity and underweight were positively associated, and frequent outdoor activity was also negatively associated with pre-DM odds.

Table 2-4 Multivariable-adjusted odds ratios for diabetes and pre-diabetes in pulmonary tuberculosis patients

Variables	Diabetes OR(95% CI)	P value	Prediabetes OR(95% CI)	P value
Age(years)				
<30(reference)	--	--	--	--
30~	5.35 (2.47-11.57)	<0.001	3.61 (1.96-6.64)	<0.001
40~	9.46 (4.61-19.43)	<0.001	4.29 (2.40-7.66)	<0.001
≥50	13.20 (6.71-25.96)	<0.001	5.74 (3.37-9.76)	<0.001
DM Family history	5.85 (4.44-7.69)	<0.001	1.58 (1.14-2.20)	0.006
BMI				
18.5-23.9(reference)	--	--	--	--
<18.5	1.30 (0.97-1.76)	0.082	1.38 (1.05-1.80)	0.019
≥24.0	1.08 (0.77-1.53)	0.644	1.44 (1.07-1.93)	0.015
Positive sputum smear	1.61(1.08-2.40)	0.021	1.36(0.96-1.94)	0.085
Cavity in chest X-ray	1.66(1.07-2.59)	0.025	0.76(0.46-1.26)	0.285
Frequent outdoor activity	0.63 (0.49-0.80)	<0.001	0.72 (0.58-0.89)	0.003
Yearly income(RMB)				
<2000(reference)	--	--	--	--
2000-9999	1.34 (0.94-1.92)	0.106	0.87 (0.65-1.16)	0.337
≥10000	1.65 (1.09-2.50)	0.017	0.95 (0.67-1.34)	0.768

Non significant variables: sex, marriage, smoking, alcohol drinking, educational level, involved lung field

Discussion

The present study shows that the prevalence of DM based on FPG values was higher in the PTB (6.3%) patients than in the non-TB (4.7%) controls, and PTB patients had 3.17 times the odds of having DM as non-TB subjects, when adjusted for possible confounding factors.

Our study was designed to discover the prevalence of DM and pre-DM in TB patients and estimate the odds ratios. The strength of this study is its community based setting. To avoid selection bias, we chose community based TB clinics instead of TB hospitals, which means the prevalence and odds ratios can be regarded as representative for the general population. Also, the large sample size allowed us to provide relatively precise estimates of current prevalence of DM in TB. To accurately define DM, we used primary data collected from newly-diagnosed PTB patients and also in a non-TB control group. In other related studies, either the DM prevalence in non-TB was not reported or the prevalence being calculated from secondary data with inherent biases[19; 20]. Only a few reports concerning the prevalence of DM in active TB patients in China are available until now. Zhang Q et al carried out a retrospective analysis in Shanghai pulmonary hospital from 2008–2009 and discovered 9.2% TB patients were complicated by DM [21]. Also from Shanghai, in 1997, Lin S et al reported 4.86% prevalence of DM in TB cases, DM tending to be more prevalent among TB patients in urban area and in older patients[22]. Liang Li et al reported a prevalence of 12.4% in a study based in different parts of China, primarily based on hospital data using FPG[23]. Until now, there were no community based data on the prevalence of DM among sufficiently large samples of TB patients as well as comparative data from non-TB controls from the same community collected in the same time period.

It has been reported that PTB may induce temporary hyperglycemia, which resolves with treatment[14]. Thus, over- diagnosis might take place if tested for glucose prior to initiation of TB treatment. Most of our study subjects were screened for DM 2–3 weeks after the initiation of TB treatment.

We found that the associated factors for having DM were basically the same for PTB patients as for the general population. Increasing age, family history of DM and high income were positive associated factors for DM in PTB patients. For BMI the pattern looks somewhat different. The World Health Organization's (WHO) "standard" definition for underweight, overweight, and obesity by BMI are ≤18.5 kg/m^2, 25.0 to 29.9 kg/m^2, and ≥30.0 kg/m^2, respectively[24]. But in China as in other parts of Asia, a modified criteria is more appropriate to forecast the risk of DM, dyslipidemia and the related diseases[17] and we used this criteria for the cut-off value. The severe underweight and moderate underweight categories only account for 3.4% and 3.7% of the TB patients respectively in our study, so we combined them with mild underweight accounting for totally 17.7% underweight patients in our study. Similarly, obesity only accounts for 0.7% in the PTB cases and we combined it with the overweight subgroup, together accounting for 13.9% overweight and obese PTB cases. Although the average BMI in PTB patients with DM, pre-DM and normoglycemia did not show obvious difference, in logistic regression analysis, underweight (BMI<18.5 kg/m^2) showed a significant association with pre-DM and borderline significance for DM. This finding was more or less similar to the study carried out in Tanzania [25] that reported severe underweight (BMI<16 kg/m2) among male TB patients was associated with DM (OR 2.52, p=0.004). Underweight is a risk factor for many chronic diseases such as respiratory diseases, osteoporosis, as well as DM[26] . Like in the general population, TB patients with overweight and obesity were at 1.44 fold odds of having pre-DM compared to normal weight patients. But the association was not significant for DM. However,

the proportion of overweight and obesity in patients with DM (17.0%) was higher than in patients without DM (13.4%). The association for BMI in the setting of co-morbid DM and TB is complex. While increasing the risk of DM and pre-DM, overweight and obesity is protective against TB disease[27]. Weight loss due to poorly controlled DM and metabolic de-compensation takes away this protection. Presence of TB increases risk of hyperglycaemia through stress but by causing weight loss reduces the risk. These interactions may play out differently in different individuals[28] .

The association of positive sputum smear with DM in TB patients was similar to what has been reported earlier[20][29]. In our study, patients with DM had the highest rate of positive sputum smear, which indicates high risk of infectivity and spread of TB by these patients. Presence of cavities on chest X-rays was significantly associated with comorbid DM in our study.

Over half of our DM cases (56.1%) were already diagnosed previously. This was lower than nearly all DM patients being aware in the report by Restrepo BI et al[19] and about 75% being aware of DM in the study by Liang Li et al[23]. This difference maybe because our study population was primarily from rural areas where DM awareness and screening are limited. In this context the importance of strengthening monitoring and control of DM becomes relevant. In the present study, only 10.3% of previously known DM cases had their blood glucose level tested regularly. The need for improving DM care services as well as health education about the link between TB and DM cannot be overemphasized, particularly in areas with double burden of the diseases. Our findings show that the opportunities for preventing TB among DM patients should receive greater attention by the health system. DM patients should be aware of their increased risk of active TB. They should be educated to report to TB clinics in time when suspicious TB symptoms occur in order to benefit from having early diagnosis and treatment. Furthermore,

underweight persons with DM should be paid high attention given the observed positive association in our study.

Also, updating the educational curriculum of health professionals to increase their awareness of the re-emerging association between TB and DM is urgently required. We discovered that the link of TB and DM was not prioritized in the seven TB clinics that participated in our project, three of which even did not measure the blood glucose level of the TB patients before we started this project. Within the framework of the current project, we implemented health education on the links between TB and DM for TB patients, health care providers and the lay public, the results of which will be reported later.

However, our study also has some limitations. First, since isolated hyperglycemia 2 hours after glucose loading is common among Asian patients with DM[30], we may have underestimated the prevalence of DM because an oral glucose tolerance test (OGTT) was not done for practical reasons. However, the same level of underestimation will apply for the non-TB group and thus does not change the relative odds.

In Shanghai Diabetes Study, 48.6% of patients with newly-diagnosed DM had isolated hyperglycemia 2 hours after glucose loading [31] . So, the actual prevalence rates of DM and pre-DM in our study may have been much higher. This may also explain the difference between our findings and other studies carried out where dual burden of TB and DM also exists. The prevalence rates of DM and pre-DM were 25.3% and 24.5% respectively among TB patients in South India[20].

Another lacuna in our study is that M. tuberculosis (MTB) infection was not confirmed by sputum culture in a substantial number of participants in the clinical settings in our study. However, according to the recent national TB epidemiological survey in China, positive MTB culture is documented in only

26.4% of the active PTB patients [32] , which means that nearly three quarter of the active PTB may be missed if we would take the MTB culture result as the gold standard. As with most TB centres, clinical manifestations, sputum smear microscopy and chest radiographs were the central component for PTB diagnosis in our study.

Our study provides further evidence of the links between DM and TB and the evidence that this interaction is playing out even in the rural settings in China where the presumed burden of DM appears low. Our findings indicate the potential benefits of partially integrating TB and DM prevention and control systems, and males may benefit the most given the higher prevalence of TB among them. Given the growing huge burden of DM in China and the high rate of undetected DM, systematic screening of all TB patients for DM through the infrastructure for TB control could serve to improve the early detection of DM, particularly in developing countries. Liang Li et al indicate that such screening has the potential of identifying almost 30,000 new cases of DM each year in China [23]. Follow-up studies are needed to identify the causal mechanism of the link between TB and DM in the future.

Acknowledgments

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3 Hyperglycemia is associated with increased risk of patient delay in pulmonary tuberculosis in rural areas

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Abstract

Background: Excessive time between the first presentation of symptoms of pulmonary tuberculosis (PTB) and diagnosis contributes to ongoing transmission and increased risk of infection in the community, as well as to increased disease severity and higher mortality. People with type 2 diabetes mellitus (T2DM) have a higher risk of developing PTB. However, the effect of T2DM on delayed diagnosis of PTB is not fully understood. This study investigated the effects of hyperglycemia (diabetes and pre-diabetes) and other factors on PTB patient delay in a rural area of China.

Methods: In the present community-based investigation, PTB patients aged ≥16 years newly diagnosed at county tuberculosis dispensaries were recruited consecutively between September 2011 and December 2013. Fasting blood glucose was determined in all subjects, and a structured questionnaire was used to collect basic information.

Results: Of the 2280 patients, 605 (26.5 %) had hyperglycemia. The median (inter-quartile range) time to seeking health care was 44 (59) days. Health care seeking was delayed in 1754 subjects, and hyperglycemia was independently associated with an increased probability (odds ratio 2.10; 95% confidence interval 1.49–2.97) of patient delay in subjects aged ≥30 years. Other factors associated with patient delay were cough, night sweats, and lack of knowledge regarding typical tuberculosis symptoms. The onset of hemoptysis was negatively correlated with patient delay.

Conclusions: Patient delay appears to be a serious problem in this rural area with a high prevalence of tuberculosis. Hyperglycemia is independently associated with an increased probability of patient delay, which, in turn, may result in more serious clinical manifestations.

Key words: community, determining factor, diabetes mellitus, infectious disease, odds ratio

Introduction

Tuberculosis (TB) continues to be a global health problem. In 2014, the worldwide incidence of TB was 9.6 million and it killed approximately 1.5 million people[1]. Early diagnosis and immediate initiation of treatment play a key role in TB control, especially in arresting TB transmission within a community. Most transmissions occur between the onset of cough and initiation of treatment. At the individual level, a delay in diagnosis is associated with poor disease prognosis[2].

Type 2 diabetes mellitus (T2DM) triples the risk of developing TB[3], and comorbidity of DM and TB represents a double burden with significant public health implications. In 2011, a collaborative framework for the joint

care and control of TB and DM was proposed by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease. China has one of the highest prevalences of TB, with an incidence of nearly 7.07 per 10000 people, accounting for 10% of all cases worldwide[1]. Although progress has been made in TB control, the results of the fifth national TB survey in China in 2010 showed that the prevalence of TB declined only minimally by 7 per 100000 people in 10 years[4]. At the same time, China has witnessed an escalating epidemic of DM[5], with the prevalence of DM and pre-DM reaching 9.7 % and 15.5 %, respectively, in 2010-11[6]. Consequently, the possibility of an interaction between DM and TB has increased in recent years. In a rural area in China, the prevalence of DM in TB patients was 6.3 %, with TB patients having a higher odds ratio (OR; 3.17) of having DM than non-TB controls[7]. It has been reported that DM is associated with more serious clinical presentations and adverse treatment outcomes for pulmonary TB (PTB)[8]. Delays in diagnosing PTB in patients with DM may present an even greater challenge for the prevention and control of PTB, with a consequently worse condition of patients.

Factors related to health system delays, including health facilities, diagnostic procedures, and disease management, vary according to countries or regions[9]. Based on our data, the median health system delay for PTB was 3 days, which may be due primarily to the considerable improvement in TB clinics in China in recent years. Therefore, in the present study, we focus on the patient delay in PTB.

Until now, there has been little information available regarding the effect of DM on diagnostic delays of TB. In a cross-sectional study conducted in two TB dispensaries in Beijing[10], DM was associated with a longer delay in diagnosis, which was, on average, 19 days (OR 3.10; 95 % confidence

interval 1.66 - 5.76). Malbasa and Pesut reported that DM was associated with a higher patient delay (>30 days) in the setting of intermediate-to-low TB incidence[11]. However, whether DM will increase the risk of a delay in seeking health care for TB in the setting of a high incidence of TB has not been reported. In China, county-level TB clinics play an important role in TB diagnosis and treatment in the community. Therefore, the present large-scale epidemiological study was undertaken using primary data to identify the association between hyperglycemia (including DM and pre-DM) with health seeking delays in PTB patients at the community level in a high TB incidence setting in a rural area in China.

Methods

Study population

The present cross-sectional study consecutively recruited patients aged ≥16 years who had been diagnosed with active PTB at the county TB center between September 2011 and December 2013. Patients who met these inclusion criteria and agreed to participate in the study were interviewed using a pretested questionnaire. Briefly, newly diagnosed PTB patients who registered for Directly Observed Treatment, Short Course (DOTS) treatment for TB from seven TB clinics (Yishui, Yinan, Lanshan, Cangshan, Tancheng, Feixian, and Pingyi TB clinics) in Linyi, China, were included in the study. Patients positive for HIV and subjects who had experienced trauma in the past 3 months or had cancer or severe cardiac, hepatic, or kidney disease were excluded from the study.

The present study was approved by the Ethics Committee of Qingdao Disease Prevention and Control Centre and was conducted according to the guidelines laid down in the Declaration of Helsinki. Written informed

consent was obtained from all subjects prior to their inclusion in the study. The study is registered with the Chinese Clinical Trial Registry (No. ChiCTROCC-10000994).

Diagnosis of DM and pre-DM

Patients reporting a prior history of DM by confirmed diagnosis or antidiabetic drug treatment were classified as being known diabetics. The screening of DM and pre-DM was based on WHO criteria for the classification of glucose tolerance based on fasting plasma glucose (FPG)[12].After an overnight fast, venous blood was collected from each participant and the glucose oxidase method was used to estimate FPG levels shortly after blood had been drawn. Subjects with FPG≥6.1 mmol/L were referred to DM clinics for diagnostic confirmation with a second FPG test. Those with FPG levels in the range 6.1 - 6.9 mmol/L were diagnosed as pre-DM and those with FPG levels ≥7.0 mmol/L were diagnosed as DM.

Definition of patient delay

The health seeking interval was taken as the time from the appearance of major pulmonary symptoms of the disease until an individual's first visit to a county TB dispensary. In the present study, a health seeking interval > 28 days was defined as patient delay.

Data collection

A pretested standardized questionnaire was used for data collection. A face-to-face interview was conducted by investigators who had received prior training. Information regarding demographic characteristics (age, gender, occupation [peasant, manual laborer, white-collar worker, retired or unemployed], marital status [married, widowed/divorced, or single], yearly household income, and education level) was collected. In addition, information was collected regarding the clinical features of PTB, such as typical symptoms (cough, hemoptysis, fever, night sweats, sputum production, chest pain, fatigue, loss of appetite), and chest X-ray results (the presence of cavities), knowledge of TB-related symptoms (cough or sputum production, hemoptysis, fever or night sweats, chest pain), and the attitude towards TB (e.g. Do you know that TB is curable? Do you feel extremely upset about the disease? Will you maintain strict secrecy of suffering the disease?).

Statistical analysis

Data were analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Data are shown as the number of cases, as the mean±SD, or as median values with the interquartile range (IQR) in parentheses. Categorical variables were compared using the Chi-squared test, whereas continuous variables were compared using t-tests or the Wilcoxon test. Multivariate logistic regression (MLR) was used to evaluate risk factors for patient delay. All factors associated with the outcome in univariate models($P<0.05$) were checked in the multivariate model, including the distance from home to the TB dispensaries, even though no statistical significance existed in the univariate model. Backward stepwise logistic regression, with entry and removal criteria of $P<0.05$ and $P>0.10$ respectively, was used to establish the

final multivariate predictive model. The model was significant (χ^2=153.983, P<0.001). The goodness of fit of the multivariate model was assessed using the Hosmer-Lemeshow test, and the fitness was good (χ^2 =4.089, P=0.769). Odds ratios and nominal 95 % confidence intervals (CIs) are presented. Two-sided P<0.05 was considered significant for all analyses.

Results

From September 2011 to December 2013, 2403 PTB patients were eligible for inclusion in the study. Of these patients, 112 patients refused to participate and 11 patients were excluded because their TB diagnosis was not confirmed. This left 2280 patients for analysis.

Table 1 lists the basic characteristics of the participants stratified by patient delay (Group 1, >28 days; Group 2, ≤28 days). Patients in Group 1 were significantly older than those in Group 2. Of the 1754 TB patients in Group 1, 515 (29.4 %) had hyperglycemia, which was significantly higher than the proportion of TB patients in Group 2 who had hyperglycemia (17.1 %; P<0.001; Table 1). With regard to the onset of typical TB symptoms, the proportion of patients reporting cough, fever, and night sweats was higher in Group 1 than in Group 2 (P < 0.001 for all), whereas fewer patients in Group 1 than Group 2 reported hemoptysis (13.7 % vs 20.9%, respectively; P < 0.001). Low education levels and a lack of knowledge about TB symptoms were more common in Group 1. There were no significant differences in chest pain, fatigue, loss of appetite, gender, body mass index (BMI), yearly income, distance from home to TB clinics, smoking, and alcohol consumption between the two groups (Table 1;P> 0.05). Although there was a tendency for sputum production and the presence of cavities, as revealed

by chest computed tomography, to be higher in Group 1 than Group 2, the differences did not reach statistical significance (Table 3-1).

Crude and adjusted ORs for patient delay are presented in Table 3-2. In the MLR models, hyperglycemia was significantly associated with an increased probability of patient delay (OR 2.053; 95 % CI 1.506 - 2.798). Of the TB-related symptoms, cough had the strongest association with patient delay (OR 6.111; 95 % CI 3.670- 10.175), followed by night sweats (OR1.933; 95% CI 1.432 - 2.608). Hemoptysis was negatively associated with patient delay (OR 0.634; 95% CI 0.460- 0.875). Other risk factors for patient delay included age ≥50 years and a lack of knowledge of typical TB symptoms.

Because age distribution in the two groups was significantly different and age is also closely related to the incidence of diabetes, we analyzed the correlation of hyperglycemia with patient delay stratified by age. The results of the MLR model after adjustment for cough, sputum production, hemoptysis, fever, night sweats, the presence of cavities, marriage status, education level, knowledge of typical TB symptoms, BMI, smoking, attitude towards TB, loss of appetite, and distance from home to TB clinics revealed that hyperglycemia was significantly related to patient delay in the age subgroups ≥ 65, 50 - 64, and 30 - 49 years. Combining these three subgroups, we found that hyperglycemia was independently associated with a nearly twofold higher probability of patient delay in participants aged ≥ 30 years (OR 2.105; 95 % CI 1.491 - 2.973; Table 3-3).

Table 3-1. Characteristics of the study subjects according to patient delay

(Group 1, >28 days; Group 2, ≤ 28 days)

	Group1	Group 2	P value
No. (%)	1754 (76.9)	526 (23.1)	
Age(years), median(IQR)	53.00(31.00)	45.00(35.00)	<0.001
Age(years)			
≥ 65	450(25.7%)	98(18.6%)	<0.001
50~64	547(31.2%)	125(23.8%)	
30~49	398(22.7%)	140(26.6%)	
< 30	359(20.5%)	163(31.0%)	
Men	1303(74.6%)	378(72.1%)	0.262
BMI(kg/m^2), mean ± SD	20.69±3.07	20.76±3.31	0.159
BMI(kg/m^2)			
< 18.5	296(16.9%)	98(18.6%)	
18.5~23.9	1297(73.9%)	368(70.0%)	
≥ 24.0	161(9.2%)	60(11.4%)	
Symptoms			
Cough	1659(97.4%)	445(87.3%)	<0.001
Hemoptysis	231(13.7%)	99(20.9%)	<0.001
Fever	822(48.6%)	184(38.8%)	0.001
Night sweats	619(36.5%)	113(23.9%)	<0.001
Sputum production	1176(67.7%)	320(63.1%)	0.054
Chest pain	370(21.9%)	119(25.4%)	0.113
Presence of cavities	226(13.9%)	50(10.5%)	0.054
Fatigue	611(35.6%)	157(32.1%)	0.147
Loss of appetite	377(22.2%)	91(19.2%)	0.169
Yearly income(CNY)			
< 10000	869(59.8%)	278(63.6%)	0.153
≥ 10000	584(40.2%)	159(36.4%)	

Hyperglycemia[1]	515(29.4%)	90(17.1%)	<0.001
Diabetes	135(7.7%)	22(4.2%)	
Impaired fasting glucose	380(21.7%)	68(12.9%)	
Distance to TB clinic(km), median (IQR)	17.90(16.90)	18.75(19.55)	
Distance to TB clinic(km)			
> 27.1	433(24.7%)	156(29.7%)	0.115
18.7~27.1	421(24.0%)	112(21.3%)	
9.8~18.6	442(25.2%)	121(23.0%)	
< 9.8	458(26.1%)	137(26.0%)	
Marital status			
Married	1370(79.3%)	375(73.1)	0.002
Widowed or divorced	80(4.6%)	21(4.1%)	
Single	278(16.1%)	117(22.8%)	
Low education level[2]	975(56.3%)	241(46.6%)	<0.001
Former or current smoker	460(26.2%)	147(27.9%)	0.432
Alcohol consumption[3]	173(9.9%)	61(11.6%)	0.250
Knowledge of TB-related symptoms[4]			
None	132(7.8 %)	21(4.4 %)	0.031
1+	540(31.8%)	168(35.0%)	
2+	442(26.0%)	129(26.9%)	
3+	369(21.7%)	90(18.8%)	
4+	214(12.6%)	72(15.0%)	
Attitude towards TB[5]			
None	103(5.9%)	37(7.1%)	0.452
1+	90(5.2%)	20(3.9%)	
2+	298(17.2%)	94(18.1%)	
3+	1245(71.7%)	367(70.8%)	

Unless indicated otherwise, data are given as n (%).

[1]Includes both diabetes and impaired fasting glucose. [2]Low education level was defined as education to primary school level or lower. [3]Alcohol consumption

means drinking alcohol at least twice a week for no shorter than one year. [4]Knowledge of tuberculosis (TB)-related symptoms was evaluated using a questionnaire regarding typical TB-related symptoms and rated as follows: none, no knowledge of TB-related symptoms; 1+, knowing one of the typical TB-related symptoms; 2+, knowing two typical TB-related symptoms; 3+, knowing three typical TB-related symptoms; 4+,knowing all typical TB-related symptoms. [5]Attitudes towards TB were evaluated using a questionnaire and rated as follows: none, no right answer to this question on the questionnaire; 1+, YES; 2+, NO; 3+, NO.

BMI, body mass index; IQR, interquartile range.

Table 3-2. Risk factors related to patient delay as determined by logistic regression analysis

	Crude		Adjusted	
	OR(95 % CI)	P-value	OR(95 % CI)	P-value
Age(years)				
≥ 65	2.08(1.67~2.7)	<0.001	2.01(1.22~3.30)	0.006
50~64	1.99(1.52~2.59)	<0.001	1.78(1.12~2.80)	0.01
30~49	1.29(0.99~1.69)	0.06	1.48(0.96~2.27)	0.07
<30 (ref.)	-	-	-	-
Male gender	1.13(0.91~1.41)	0.26	/	/
Body mass index(kg/m²)				
< 18.5	0.88(0.66~1.11)	0.23	/	/
≥ 24.0	0.76(0.55~1.05)	0.09	0.63(0.42~0.94)	0.02
18.5~23.9 (ref.)	-	-	-	-
Symptoms				
Cough	5.38(3.63~7.96)	<0.001	6.11(3.67~10.17)	<0.001
Hemoptysis	0.59(0.46~0.77)	<0.001	0.63(0.46~0.87)	0.006
Fever	1.48(1.20~1.83)	<0.001	/	/
Night sweats	1.83(1.45~2.31)	<0.001	1.93(1.43~2.60)	<0.001
Sputum production	1.22(0.99~1.50)	0.05	/	/

Presence of cavities	1.37(0.99~1.90)	0.05	1.41(0.96~2.06)	0.08
Chest pain	0.82(0.65~1.04)	0.11	/	/
Fatigue	1.17(0.94~1.45)	0.15	/	/
Loss of appetite	1.19(0.92~1.54)	0.17	/	/
Yearly income(CNY)				
< 10000	0.85(0.68~1.06)	0.15	/	/
≥ 10000(ref.)	-	-		
Hyperglycemia	2.01(1.56~2.57)	<0.001	2.05(1.50~2.79)	<0.001
Marital status				
Married (ref.)	-	-	/	/
Widowed or divorced	1.04(0.63~1.71)	0.86	/	/
Single	0.65(0.51~0.83)	0.001	/	/
Low educationlevel[1]	1.47(1.21~1.76)	<0.001	/	/
Alcohol consumption[2]	0.83(0.61~1.13)	0.25	/	/
Smokers	0.92(0.73~1.14)	0.43	/	/
Knowledge of TB-related symptoms[3]			/	/
none	2.09(1.23~3.57)	0.006		
1+	1.08(0.78~1.48)	0.63		
2+	1.15(0.82~1.60)	0.40		
3+	1.37(0.96~1.96)	0.07		
4+(ref.)	-	-		
Attitude towards TB[4]				
none	0.82(0.55~1.21)	0.3	2.29(1.21~4.36)	0.011
1+	1.32(0.80~2.18)	0.26	/	/
2+	0.93(0.72~1.21)	0.60	/	/
3+(ref.)	-	-	-	-
Distance to TB clinic (km)			/	/
>27.1	0.83(0.63~1.08)	0.16		
18.7~27.1	1.12(0.84~1.49)	0.41		

9.8~18.6	1.09(0.82~1.44)	0.53
<9.8(ref.)	-	-

[1]Low education level was defined as education to primary school level or lower. [2]Alcohol consumption means drinking alcohol at least twice a week for no shorter than one year. [3]Knowledge of tuberculosis (TB)-related symptoms was evaluated using a questionnaire regarding typical TB-related symptoms and rated as follows: none, no knowledge of TB-related symptoms; 1+, knowing one of the typical TB-related symptoms; 2+, knowing two typical TB-related symptoms; 3+, knowing three typical TB-related symptoms; 4+, knowing all typical TB-related symptoms. [4]Attitudes towards TB were evaluated using a questionnaire and rated as follows: none, no right answer to this question on the questionnaire; 1+, YES; 2+, NO; 3+, NO.
OR, odds ratio; CI, confidence interval.

Table 3-3. Logistic regression analysis for hyperglycemia stratified by age and the probability of patient delay

	Crude		Adjusted[1]	
	OR(95 % CI)	P-value	OR(95 % CI)	P-value
All	2.009(1.566~2.577)	<0.001	2.053(1.506~2.798)	<0.001
Age(years)				
≥65	1.964(1.157~3.334)	0.012	1.921(1.003~3.678)	0.049
50~64	2.211(1.360~3.596)	0.001	2.125(1.189~3.797)	0.011
30~49	1.613(1.016~2.560)	0.042	2.303(1.234~4.297)	0.009
<30	1.751(0.979~3.002)	0.059	1.539(0.717~3.304)	0.269
Age(years)				
≥30	1.931(1.457~2.558)	<0.001	2.105(1.491~2.973)	<0.001
<30	1.715(0.979~3.002)	0.059	1.539(0.717~3.304)	0.269

[1]Adjusted for cough, sputum production, hemoptysis, fever, night sweats, the presence of cavities, loss of appetite, body mass index, marriage status, education level, knowledge of tuberculosis (TB)-related symptoms, smoking status, attitudes towards TB, and the distance to TB clinics. OR, odds ratio; CI, confidence interval.

Discussion

In the present community-based observational study, hyperglycemia was independently associated with an in- creased probability of patient delay in subjects aged ≥30 years. Significant associations were also found for the onset of cough, night sweats, old age, and a lack of knowledge of TB-related symptoms, whereas hemoptysis was inversely correlated with patient delay.

An important challenge for TB control is inadequate and late case detection [13].People who experience signs and symptoms of TB should report promptly for formal diagnosis in TB clinics. Unfortunately, in many low-to-middle income countries that have a high TB burden [14; 15; 16], a diagnosis delay is still common. The underlying reasons reflect factors related to patients and the healthcare system. Because, as noted in the Introduction, there was no significant health system delay, we focused on patient delay in the present study.

The median (IQR) health-seeking interval was 44 (59) days, with 76.9% of patients having a health-seeking delay. In regions with a high prevalence of TB, early diagnosis is considered one that occurs 2-3 weeks after the onset of clinical symptoms and late diagnosis is considered one that occurs 4 weeks after onset [17].The patient delay in the present study was longer than that reported in some previous studies. For example, it has been reported that 47% of Chinese patients with TB symptoms seek health care in a timely manner [4], but one-third knowledge of typical TB symptoms, BMI, smoking, attitude towards TB, loss of appetite, and distance from of suspected TB cases in China's rural areas do not seek care after 3 weeks of persistent cough [18]. The longer delay in the present study may be due to different definitions of patient delay and the study populations selected for analysis. In the present study, patient delay was defined as the time between the

onset of PTB symptoms and the first visit to a county-level TB dispensary, and participants were recruited from newly diagnosed PTB patients in a rural area with a high incidence of TB. The education level of nearly half the participants in the present study was primary school or lower. We suppose that the low education level and the associated low ability to recognize TB symptoms may have contributed to the higher patient delay. Although programs such as DOTS have proven to be successful for TB control in China [19], concerns regarding the effectiveness of such programs for the most vulnerable members of society, particularly the rural poor, have been raised [20], and similar findings were made in the present study.

Individual factors, involving rural residence, old age, female gender, and low education level, have been re- ported to lead to patient delay [15; 21; 22]. However, in recent years, the coexistence of DM and TB has posed a new challenge, and a deeper understanding of the effects of DM on TB patient delay is urgently needed.

In the present study, hyperglycemia, including DM and pre-DM, was associated with a nearly twofold higher probability of patient delay. This indicates that DM may facilitate the transmission of TB within the community. In addition, because diagnosis delay is closely related to a more severe clinical presentation and poorer prognosis, this association further aggravates the disease burden at the level of the individual. In terms of underlying mechanisms, the risk of being afflicted with infectious disease increases significantly in DM patients who are immunocompromised, which includes impaired neutrophil bactericidal function, cellular immunity, and complement activation caused by hyperglycemia. It has been reported that the incidence of infection in DM is in the range 35.0 % - 90.0 % [23], with lower respiratory tract infections being one of the most common

complications. We suppose that frequent symptoms of cough, fever, and chills that are seen in lower respiratory tract infections overlap typical TB manifestations, which may explain, in part, why patients with hyperglycemia are prone to have a longer health-seeking delay. Stratified analysis revealed that this association was present in patients older than 30 years of age. One possible explanation is that the chances of suffering from hyperglycemia are quite low in people younger than 30 years of age.

Similar to other studies [15; 21], in the present study we observed that old age, low education level, the onset symptoms of cough and night sweats, and a lack of knowledge of TB-related symptoms were associated with patient delay. However, we did not find an association between rural residence and patient delay, as reported by others [22], and this may be due to the significantly improved transportation in the area investigated in the present study.

To avoid selection bias, we chose to recruit subjects from community-based TB clinics instead of TB hospitals, which means the existence of patient delay and the risk factors can be regarded as representative of the general population. This is the main strength of the present study. In addition, the large sample size allowed us to provide relatively precise estimates of the effects of hyperglycemia on patient delay. To define the association more accurately, we used primary data collected from newly diagnosed PTB patients.

A major limitation of the present study is that the definition of the onset of TB symptoms relied on self-report, which may have led to recall bias. However, the diagnostic procedures in this area were uniform and standard diagnostic methods were used. The socio-demographic variables, including income, education, and type of health facility consulted by TB patients, were similar across participants. Another weakness of the present study may be

that we did not perform 2-h oral glucose-tolerance tests (OGTTs). Most of the participants lived in rural areas and often had a long way to travel to get to the county TB clinics, which made impractical to perform OGTTs. This may have led to an underestimate of the prevalence of diabetes. However, we included patients with pre-DM in the present study, which may compensate, in part, for the possibly lower number of TB patients.

The present study provides the first evidence that hyperglycemia, including DM and pre-DM, is independently associated with an increased risk of patient delay in people aged ≥30 years in a rural area with a high incidence of TB. The findings indicate that screening for TB in DM patients, especially those aged≥30 years, may contribute significantly to reduce the transmission of TB through early diagnosis. The mechanism underlying the effects of hyperglycemia on TB patient delay needs further investigation.

Acknowledgments

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4

Severe hypovitaminosis D in active tuberculosis patients and its predictors

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Abstract

Background & aims: Tuberculosis (TB) patients have a significant vitamin D deficiency (VDD) endemic, which may be closely related to the onset and progress of the disease. The comorbidity of diabetes (DM) and TB has posed an increasing challenge in recent years. However, the influence of DM on TB and the possible mechanism are still uncertain. We carried out this study to identify the nutritional status of vitamin D (VD) in TB patients in a northern city in China (latitude 36°N) and investigate the possible predictors of severe vitamin D deficiency (SVDD).

Methods: A cross-sectional study including 461 active TB patients (192 with and 269 without DM) was randomly selected from Qingdao Chest Hospital from June 2015 to August 2016. We measured serum 25-hydroxyvitamin D [25(OH)D], and investigated the association between socio-demographic, dietary intake, DM, body mass index (BMI), severity of initial TB signs and

symptoms (TB score) and VD status. Multivariate logistic regression analysis was used to define the possible predictors of SVDD.

Results: The median serum 25(OH)D concentration was 8.50 ng/mL. Of the 461 TB patients included, 383(83.1%) had VDD [25(OH)D < 20 ng/mL], and 217 (47.1%) had SVDD [25(OH)D<8 ng/mL]. The variables associated with serum 25(OH)D concentrations were DM, outdoor activity level, TB score and BMI (p < 0.05). Patients with severe TB score had nearly 5 fold higher risk of having SVDD compared with those in mild subgroup [OR (95% CI) 4.919 (2.644 - 9.150), p < 0.001]. Low outdoor activity level also increased the odds of SVDD, while DM and high fish consumption showed protect effects.

Conclusions: Severe hypovitaminosis D is prevalent in active TB patients, and the main predictors of SVDD were severe TB score, low outdoor activity, inadequate fish consumption. Lowered serum 25(OH)D may be associated with increased risk of TB in DM.

Introduction

Vitamin D deficiency (VDD) is a worldwide public health problem that is the cause of rickets, but is also associated with a range of common chronic diseases including diabetes, infection, cardiovascular disease, autoimmune diseases and cancer. Mainly due to new findings of the functions of this sunshine vitamin, a surge of research investigating the relationship between vitamin D (VD) and tuberculosis (TB) started in the 21st century [1; 2; 3; 4; 5; 6; 7; 8]. Exposure of TB patients to sunlight, which induces cutaneous VD synthesis, became common practice already in the early 20th century [9]. However, in the middle of the 20th century, anti-tuberculosis drugs like rifampin, isoniazide, pyrazinamide were introduced and VD lost its role in therapy. It

has been reported that in activated macrophages and T lymphocytes, the main effectors of anti-TB immunity in the body, there exist receptors of the active form of vitamin D [1,25(OH)$_2$D3] [10], which was discovered to be important in the congenital antimicrobial response pathway in macrophages [11]. Serum 25-hydroxyvitamin D[25(OH)D] is the most reliable index of vitamin D (VD) status in the body [12]. VDD [serum 25(OH)D < 20 ng/mL] and severe vitamin D deficiency (SVDD) [13] [serum 25(OH)D < 8 ng/mL] were reported in TB patients [2; 3; 4; 13; 14], even in equatorial countries like Tanzania [2]. A systematic review showed that up to 88.9% patients with TB had VDD and the main predictors were lack of ultraviolet exposure, inadequate dietary intake, comorbidities and old age [5].

In recent years, the double burden of increasing prevalence of diabetes mellitus (DM) in addition to endemic city of TB in the developing world, has posed an increasing challenge to public health care. According to the global tuberculosis report 2016 (WHO), the TB epidemic was greater than previously estimated, with a total of 10.4 million new cases in 2015, and 1.4 million deaths attributable to TB. Although a downward trend of TB incidence has been observed from 2000 to 2015, China still accounts for nearly 8.8% of the world's TB burden, with an estimated 918 thousand new cases in 2015 [15]. In the meantime, the country has also witnessed an escalating incidence of DM. The age standardized prevalences of DM and impaired glucose tolerance (IGT) have reached 9.7% and 15.5% respectively [16], and a 4-year cumulative incidence of DM of 11.8% was reported in Qingdao [17], our research center. Epidemiologic studies showed that DM patients had a higher risk of active TB [18; 19]. Similarly, we reported that compared with non-TB controls, TB patients had a higher odds of DM (adjusted OR 3.17) in a large scale epidemiologic study [20]. A possible underlying mechanism is that patients with DM have impaired immune responses due to depressed macrophage and lymphocyte function, which in

turn may facilitate active TB [21]. Although there still exist discrepancies [22], low circulating 25(OH)D has been widely reported to be correlated with impaired insulin secretion and insulin action among diabetic patients [23; 24; 25; 26]. However, few studies have looked into the association of DM with vitamin D status or VDD prevalence in active TB.

Therefore, we carried out this study to evaluate vitamin D nutritional status in active TB patients, investigating the role of DM, and exploring the main predictors of circulating 25(OH)D and SVDD.

Methods

Design and participants

In this cross-sectional study, 461 active TB patients aged 16 - 86 years and registered for Directly Observed Treatment, Short Course (DOTS) were randomly selected from Qingdao Chest Hospital from June 2015 to August 2016, including 192 TB with DM (TB+DM) and 269 TB without DM (TB-DM). The hospital is located in Qingdao, Shandong province of China which has a catchment area of about 9 million people, and the average number of hospitalized patients with TB was about two thousand cases per year. TB was diagnosed based on chest radiography, sputum smear microscopy and clinical manifestations according to the standard tuberculosis diagnosis criteria. DM was defined on the basis of self-reported previous diagnosis of DM, the use of either insulin or oral hypoglycaemic drugs, or FPG (fasting plasma glucose) ≥7.0 mmol/L in admission biochemical test. Patients with AIDS, cancer, severe cardiac, hepatic or renal diseases, trauma in the last three months, or those taking vitamin supplements in the previous 6 months were excluded.

The Ethics Committee of Qingdao Disease Prevention and Control Centre approved the present study, and all the participants provided written informed consent. This investigation research was registered in the Chinese Clinical Trial Registry (No. ChiCTR-IPR - 15006395).

Sample size calculation

We estimated the number of TB patients recruited in this investigation by using a power calculation based on a study from Korea, a country at similar latitude, reporting 51.5% VDD in TB patients [3]. The number of tuberculosis patients needed was 420. An estimated drop-out rate of 10% was taken into account.

Questionnaire and anthropometrical measurements

Structured questionnaires were used by trained interviewers to collect information on demographic variables (including age, gender, educational level, marital status, address, occupation and ethnicity), medical history, dietary intake and lifestyle. For dietary intake, 3 day 24-h dietary recall and semi-quantitative Food Frequency Questionnaire (SQ-FFQ), a valid tool to assess habitual dietary intake were used. The SQ-FFQ used in the present study consisted of food items known to contribute to dietary VD intake and was modified according to local dietary pattern. Fish, milk, meat (pork, beef, mutton, chicken and duck) and eggs were included. Fish and meat intakes were assessed overall. Lifestyle included smoking, alcohol consumption and outdoor activity level. Individuals who had smoked ≥100 cigarettes in previous years or continued smoking were considered as smokers. Those who consumed alcohol ≥1 cup/30 days (half a pint of beer, 125 g wine or 40 g spirits) regularly in the past or continued drinking alcohol were considered as alcohol consumers. Outdoor activity level was assessed based on self-reported outdoor exercise time including running, jogging, walking, swimming, bicycling, dancing in public square, etc for the purpose to

estimate sunlight exposure time, and the results were categorized into <2 h/day and ≥2 h/day.

Height and weight were measured according to a standardized procedure with the patient in light clothes, without shoes and with an empty stomach between 6.30-8.30 am. We used the formula BMI= weight (kg)/[height (m)^2 to calculate body mass index (BMI). The cut-off value of BMI for Chinese population (<18.5, 18.5-23.9, ≥24) was used for the classification of underweight, normal weight, overweight and obesity, respectively.

Severity categories of initial TB clinical manifestation

Based on signs and symptoms of TB including cough, haemoptysis, dyspnoea, chest pain, night sweating, anaemia, tachycardia, lung-auscultation finding, fever(axillary temperature>37℃), and BMI before treatment, we assessed initial TB clinical manifestation using TB score [27; 28]. The patients were divided into three severity categories: mild, moderate and severe with a TB score of 0-2, 3-4 and >4 points, respectively (Table 4-1). The signs and symptoms were extracted from the patient records.

Table 4-1. Parameters used for TB score

Parameters	Points assigned
Self-reported	
Cough	1
Haemoptysis	1
Dyspnoea	1
Chest pain	1
Night sweating	1
Anaemia	1
Tachycardia	1
Lung-auscultation finding	1
Fever[a]	1

BMI <18	1
BMI <16	1

Note: BMI, body mass index. The parameters used for calculating TB score, including cough, haemoptysis, dyspnoea, chest pain, night sweating, anaemia, tachycardia, lung auscultation finding, fever, BMI <18, BMI <16 were shown. [a] Fever, axillary temperature >37.0℃.

Laboratory analysis

Blood samples were drawn from each subject after fasting for at least 12 h and an overnight rest. Glucose and lipid indexes were determined immediately after blood was drawn. The remaining serum samples were stored at 80℃ until measurement for 25(OH)D.

Serum 25(OH)D concentration was measured as described [29] in Beifang Institute of Biotechnology (Beijing, China). Briefly, ^{125}I radioimmunoassay (RIA) kit from DiaSorin Inc (Stillwater, Minnesota, USA) was used. The 25(OH)D assay was a two-step procedure. First, 25(OH)D and other hydroxylated metabolites from serum were rapidly extracted. Then, the treated sample was assayed based on an antibody with specificity to 25(OH)D using an equilibrium RIA procedure. As claimed by the company DiaSorin, the kit antibody would demonstrate some cross-reactivity with all forms of dihydroxyvitaminD$_2$ and D$_3$ steroids; however, in humans, these compounds were naturally present only in picomolar concentrations. For quality control, two reference controls provided with the kit were utilized (one at low-normal level and one at high-normal level) to guarantee assay performance. The sensitivity of the assay was 1.5 ng/mL. The interassay variability was 10.5% and the intra- assay variability was 8.2%.

Glucose oxidase method was used for the determination of FPG using a Hitachi automatic analyzer. Lipid indexes including total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLC) were determined by autoanalyser under standard procedure.

The following cut-off values were used for assessing vitamin D status: serum 25(OH)D concentrations < 8 ng/mL (<20 nmol/L) were classified as severe vitamin D deficiency (SVDD); 25(OH) D <20 ng/mL (<50 nmol/L) as VD deficiency (VDD); 25(OH)D of 20 - 30 ng/mL (50 - 75 nmol/L) as VD insufficiency; and those who had serum 25(OH)D 30 ng/mL (≥75 nmol/L) were VD adequate [13; 30].

Statistical analysis

Continuous data were expressed as mean ± standard deviation or median and interquartile range (IQR) and compared using independent T test or non-parametric Mann-Whitney U test. For categorical variables, proportions were reported and Pearson's χ^2 test was used for the comparisons. Univariable analysis of factors associated with serum 25(OH)D concentrations was carried out using Pearson's or Spearman's correlation analysis and univariate linear regression analysis. Further, multiple linear regression was used. Univariate and multivariate logistic regression analyses were used to calculate odds ratios of SVDD for age (year), sex (male, female), DM (yes, no), BMI (kg/m2), TB score (mild, moderate and severe), outdoor activity (<2 h/day, 2 h/day), consumption of foods providing VD (fish, milk, meat, egg) (as categorized variables), smoking (yes, no), alcohol consumption (yes, no), season of blood sampling (Spring: March-May; Summer: June-August; Autumn: September- November; Winter: December-February). Factors found to be significant in the univariate model were analyzed as independent variables or covariates in the multiple models.

The variables in multivariate analyses were included in the final models if p < 0.05 and excluded when p > 0.10. BMI, age and season were known to affect vitamin D status in the body, therefore, these covariates were adjusted in both multivariate models, and co-linearity diagnosis among the variables was performed prior to the analysis. All the analyses were performed using SPSS version 21.0 software (IBM SPSS Statistics 21); statistical significance was defined as p < 0.05.

Results

Serum 25(OH)D concentrations in active TB patients

A left skewed distribution of serum 25(OH)D was observed in the total population (n=461). The maximum concentration was 75.85 ng/mL, with a median of 8.50 ng/mL and an interquartile range of 12.49 ng/mL. 83.1% of the participants had VDD (n= 381/461), and 47.1% had SVDD (n=217). TB patients with DM had a significantly higher concentration of serum 25(OH)D compared with those without DM (Median, IQR 10.58, 13.71 vs 7.26, 12.17 ng/mL, p=0.002). TB with DM patients had a significantly lower proportion of SVDD (38.5% vs 53.2%, p < 0.05).

General characteristics of the participants by vitamin D status

Basic characteristics of SVDD (n =217) and non-SVDD (n =244) subjects are presented in Table 4-2. There were no significant differences in age, SBP, DBP, BMI, FPG, TG, LDLC between the two groups (p > 0.05). Also, the proportions of males, milk consumption, smoking, alcohol drinking, and season of blood sampling categories showed no significant differences (p > 0.05). SVDD patients had a significantly lower prevalence of DM than

non-SVDD (34.1% vs 48.4%, p < 0.01), but a higher percentage of severe and moderate TB score (p < 0.001). The proportion of patients in the highest quartile of fish consumption in non-SVDD was greater than SVDD (p < 0.05). Also, a higher proportion of outdoor activity ≥2 h/day was observed in non-SVDD (44.7% vs 25.8, p < 0.001).

Table 4-2. Characteristics of the participants by vitamin D status ($\bar{x}\pm s$)

	SVDD	Non-SVDD	P-value
N	217	244	
Age(years)	46.23±17.06	47.60±16.91	0.388
Male, n(%)	166(76.5)	175(71.7)	0.243
SBP(mmHg)	124.57±17.11	121.50±15.94	0.104
DBP(mmHg)	77.32±11.42	75.14±9.79	0.074
BMI(kg/m²)	21.64±3.08	21.43±3.43	0.508
DM	74(34.1)	118(48.4)	0.002
FPG(mmol/L)[a]	5.32,2.65	5.81,2.75	0.834
TC(mmol/L)	4.17±1.22	4.46±1.28	0.018
TG(mmol/L)[a]	0.95,0.73	1.03,0.66	0.074
HDLC(mmol/L)[a]	1.21,0.41	1.27,0.56	0.002
LDLC(mmol/L)[a]	2.35,0.89	2.54,1.09	0.102
TBscore[b], n(%)			
Mild	50(23.0)	108(44.3)	<0.001
Moderate	98(45.2)	90(36.9)	
Severe	69(31.8)	46(18.9)	
Fish consumption(g/d)			
<10	60(27.6)	52(21.3)	0.003
10~	76(35.0)	62(25.4)	
20~	40(18.4)	51(20.9)	

≥42.86	41(18.9)	79(32.4)	
Milk consumption(g/d)			
<50	37(17.0)	49(20.2)	0.292
50~	64(29.5)	49(20.2)	
100~	74(33.9)	105(43.1)	
≥200	42(19.6)	41(16.5)	
Egg consumption(g/d)			
100	30(14.0)	39(16.1)	0.018
50	163(75.2)	148(60.5)	
<50	24(10.9)	57(23.4)	
Meat consumption(g/d)			
<77.85	49(22.5)	67(27.3)	0.697
77.85~	56(25.6)	59(24.2)	
164.29~	60(27.9)	55(22.7)	
≥313.81	52(24.0)	63(25.8)	
Smoking			
Yes	111(51.1)	122(50.0)	0.855
No	106(48.9)	122(50.0)	
Alcohol drinking			
Yes	71(32.6)	91(37.4)	0.412
No	146(67.4)	153(62.6)	
Season			
Spring	57(26.3)	52(21.3)	0.535
Summer	55(25.3)	59(24.2)	
Autumn	50(23.0)	66(27.0)	
Winter	55(25.3)	67(27.5)	
Outdoor activity			
<2hs/day	161(74.2)	135(55.3)	<0.001
≦2hs/day	56(25.8)	109(44.7)	

SVDD, severe vitamin D deficiency; non-SVDD, severe vitamin D deficiency; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDLC, high density lipoprotein

cholesterol; LDLC, low density lipoprotein cholesterol. The basic characteristics of the subjects by vitamin D status (SVDD, non-SVDD) were shown. Number of cases, age, gender, SBP, DBP, BMI, DM, FPG, lipid indexes, TBscore, fish, milk, egg and meat consumption, smoking, alcohol drinking, season ofthe blood collection and outdoor activity in each group were presented.

[a] Skewed distribution was observed and median, interquartile range were used for the description. [b] TB-score, mild 0~2 points, moderate 3~4 points, severe >4 points.

The predictors of serum 25(OH)D concentrations

Correlation analysis showed that BMI was negatively associated with Log10 25(OH)D (r =-0.119, p= 0.013). TB score was negatively related with serum 25(OH)D (r =-0.270, p < 0.001), while positive associations were observed between DM, outdoor activity level and 25(OH)D. Univariate linear regression analysis showed similar results, indicating outdoor activity level and TB score may explain 10.1% and 8.5% of the variation in 25(OH)D concentrations, respectively. Multivariate linear regression analysis showed that outdoor activity level, DM, TB score, BMI were predictors for serum 25(OH)D adjusted for age and season of blood sampling. The variables included in the final model together explained 22.2% of the variation in serum 25(OH)D (R^2=0.222, p < 0.05). See in Table 4-3.

Table 4-3. The results of linear regression analysis of serum 25(OH)D (ng/mL)

	Univariable		Multivariable	
	B(95% CI)	P-value	B (95% CI)	P-value
Age[a]	0.045(-0.021~0.110)	0.180	0.017(-0.048~0.082)	0.614
Sex	0.332(-2.203~2.868)	0.797		
DM	3.189(-5.427~-0.951)	0.005	5.250(2.948~-7.551)	<0.001
BMI[a] (kg/m²)	-0.562(-0.914~-0.211)	0.002	-0.750(-1.069~-0.431)	<0.001
TBscore[b]	-4.617(3.224~6.011)	<0.001	-4.989(-6.447~-3.532)	<0.001
Season[h]	-0.067(-1.076~0.942)	0.896	-0.729 (-.663~0.205)	0.126
Outdoor activity[d]	8.036(5.835~10.237)	<0.001	5.711 (3.480~7.943)	<0.001
Fish consumption[c]	0.426(-1.418~0.566)	0.399		
Milk consumption[e]	-0.376(-1.443~0.691)	0.488		
Meat consumption[f]	-0.382(-1.264~0.499)	0.394		
Egg consumption[g]	-1.083(-2.830~0.664)	0.223		
Alcohol drinking	-0.300(-1.313~0.714)	0.561		
Smoking	-0.565(-2.502~1.371)	0.566		

DM, diabetes mellitus; BMI, body mass index.

[a]Age, BMI were analyzed as continuous variables; [b] Categorized as mild: 0 - 2 points, moderate: 3 - 4 points, severe:>4 points; [c]Categorized as <10, 10~g/d, 20~, ≥42.86 g/d; [d]Categorized as <2, ≥2 h/d. [e] Categorized as <50, 50~, 100~-,≥200 g/d; [f] Categorized as <77.85, 77.85~, 164.29~, ≥313.81 g/d. [g] The weight of an egg was estimated to be 50 g, and the egg consumptions were categorized as 100, 50 and <50 g/d; [h]Categorized as 1: March-May, 2: December-February, 3: June-August, 4: September-November.

Odds ratios for severe vitamin D deficiency among active TB patients

As shown in Table 4-4, the possible factors associated with SVDD found in univariate logistic regression analysis were DM, TB score, fish consumption and outdoor activity. Multivariate logistic regression showed a 70% decreased odds of having SVDD for DM [OR (95% CI) = 0.287 (0.175-0.470), p

< 0.001]. Severe and moderate TB score, low fish consumption and low outdoor activity were associated with increased risk of having SVDD (p < 0.05). Patients with severe TB score had nearly 5 fold higher risk of having SVDD compared with those in mild subgroup [OR (95% CI) 4.919 (2.644 - 9.150), p < 0.001]. BMI showed a borderline positive association with the odds of SVDD [OR (95% CI) = 1.062 (0.994 - 1.136, p = 0.074].

Table 4-4. Logistic regression of predictors of severe vitamin D deficiency in TB

	Univariate OR (95%CI)	P-value	Multivariate OR (95%CI)	P-value
DM	0.553(0.379~0.805)	0.002	0.287(0.175~0.470)	<0.001
TBscore[a]				
Mild				
Moderate	2.352(1.514~3.655)	<0.001	2.327(1.408~3.847)	0.001
Severe	3.240(1.962~5.350)	<0.001	4.919(2.644~9.150)	<0.001
Fish consumption (g/d)				
<10	2.449(1.439~4.169)	0.001	3.158(1.675~5.955)	<0.001
10~	2.452(1.482~4.054)	<0.001	3.995(2.195~7.272)	<0.001
20~	1.569(0.897~2.742)	0.114	1.988(1.057~3.737)	0.033
≥42.86				
Outdoor activity (hs/d)				
<2	2.321(1.564~3.446)	<0.001	2.016(1.263~3.220)	0.003
≥2				
BMI(kg/m^2)	1.020(0.963~1.080)	0.507	1.062(0.994~1.136)	0.074
Age(years)	0.995(0.985~1.006)	0.388	0.996(0.982~1.009)	0.533
Season				
Spring	1.176(0.695~1.989)	0.546	0.858(0.473~1.555)	0.613
Autumn	0.881(0.528~1.469)	0.626	0.756(0.423~1.352)	0.346
Winter	0.813(0.483~1.366)	0.434	0.684(0.375~1.248)	0.216

Summer		
Male	1.283(0.844~1.953)	0.244
Smoking	1.046(0.645~1.697)	0.855
Alcohol drinking	0.809(0.487~1.343)	0.412
Meat consumption(g/d)		
<77.85	0.882(0.440~1.767)	0.723
77.85~	1.133(0.567~2.267)	0.724
164.29~	1.321(0.661~2.641)	0.430
≥313.81		
Egg consumption(g/d)		
<50	0.536(0.218~1.321)	0.176
50	1.437(0.710~2.907)	0.313
100		
Milk consumption(g/d)		
<50	0.707(0.295~1.694)	0.436
50~	1.227(0.538~2.798)	0.626
100~	0.662(0.311~1.408)	0.284
≥200		

BMI, body mass index. [a] TB-score, mild 0 - 2 points, moderate 3 - 4 points, severe >4 points.

The result of logistic regression including univariable and multivariable models for the predictorsof severe vitamin D deficiency (SVDD) in TB patients was shown. Severe and moderate TB score, low fish consumption, low outdoor activity may increase the odds ratio of SVDD, while DM had a protective effect.

Discussion

We observed a high prevalence of VDD (83.1%) in active tuberculosis patients with a median circulating 25(OH)D concentration of 8.50 ng/mL,

which was about 50% of 25(OH)D in the general Chinese population (16.37 ng/mL) detected in our earlier study [29].

A study in an equatorial population found that mean serum 25(OH)D in TB patients was 34.64 ng/mL and the deficiency rate was 41.2% [2]. Another investigation in Korean population reported that TB patients had a lower median 25(OH)D concentration than the control (9.86 ng/mL vs 16.03 ng/mL) [3]. This result was close to our findings. From an ecological epidemiology viewpoint, we get the impression that latitude is an important factor of VD status in TB. It has been discovered that vitamin D elicits pleiotropic antimicrobial responses in vitro [31]. Coussens et al. reported that adjunctive high-dose vitamin D in TB treatment could accelerate sputum smear conversion, and reduce the inflammatory responses associated with higher risk of mortality [32]. Therefore, the common occurrence of hypovitaminosis D in active TB patients should be given full attention. Although the reasons of the high prevalence of VDD in TB patients are still uncertain, we speculate that the following aspects may be relevant. First, the synthesis of VD under skin upon exposure to sunlight, the main source of VD, may be impaired due to restricted outdoor activity of the patients. Second, severe weight loss, decreased appetite and food intake may result in nutrients depletion [33] in general and VDD in particular. Third, TB patients have obviously reduced adipose tissue, which may result in diminished VD reserves [34]. Furthermore, the requirement of VD increases in TB. Vitamin D plays a key role in anti-TB immunity, binding to toll-like receptors on macrophages and leading to increased production of 1,25(OH)2D from 25(OH)D [35], which may also contribute to lowered 25(OH)D concentrations.

Furthermore, compared with VD concentrations in DM patients(19.50 ng/mL) as reported in our earlier study [29], TB patients with DM had significantly lower serum 25(OH)D level which was approximately half that of DM. This may partially explain the positive correlation of DM with VD

status in TB patients in our study. Also, this may give us important clue of the possible consequences of hypovitaminosis D in the presence of active TB in DM. Since DM usually develops before TB in the patients with combined diseases [36], our findings indicate that significant decreasing of vitamin D status may be associated with increasing possibility of active TB in DM patients.

TB score, outdoor activity, fish consumption were also found to be associated with the prevalence of SVDD. The severity of initial TB signs and symptoms showed a negative association with serum 25(OH)D and patients with severe TB score had a nearly 5 fold higher odds of SVDD compared with those with mild TB score. This provides further evidence for the perspective that VD deficiency may be associated with TB incidence [1; 2; 3]. Also, TB is a typical chronic wasting disease, and the synthesis and bioavailability of vitamin D in the body may be impaired. Further research is needed to establish a causal relationship between vitamin D status and the incidence of active TB.

Patients who have outdoor activity <2 h/d had 2 fold higher odds of SVDD compared with ≥2 h/d. Similarly, a cross-sectional study in 782 Danish children found that outdoor walking during school hours was positively associated with 25(OH)D [37], and outdoor exercise was associated with 47% lowered possibility to have hypovitaminosis D in the obese [38]. Outdoor activity means sunlight exposure, resulting in vitamin D synthesis in the skin. Therefore, various kinds of outdoor activity suitable for TB patients should be encouraged. Fish consumption, the major source of VD from natural food, has been reported to increase concentrations of 25(OH)D [39; 40; 41; 42]. In our study, 120 (26.3%) patients in the highest quartile of fish consumption (≥42.86 g/d) had significantly lower odds of SVDD. Although there are some exceptions [43], it is often reported that vitamin D content in different species of fish differs significantly, and fatty fish may be a better VD source than

lean fish [41; 44]. In our study, total fish consumption was evaluated, which hinders further analysis of the correlation between fish species and the odds of SVDD. Nevertheless, since Qingdao is a seaside city where marine fish such as Spanish mackerel, yellow croaker, butterfish, weever, hairtail are the main species people consume, our results may provide evidence for a negative relationship between marine fish consumption and SVDD in TB.

We found BMI had a weak negative correlation with VD status with β coefficient of -0.75 and it had a borderline positive association with increased odds of SVDD. It has been reported by many researches in recent years that an inverse correlation exists between circulating 25(OH)D and BMI in the healthy population, in overweight/obese subjects and in diabetic patients [45; 46; 47; 48; 49; 50; 51]. It was speculated that low vitamin D status in obesity is due to a greater pool of distribution [52]. However, there is still insufficient evidence in TB patients, and the available data differ. Similar to our study, Friis et al. reported that TB patients with lower BMI had higher serum 25(OH)D [53]. However, an investigation in 105 TB patients found that VDD is more obvious in those with low BMI [4], and BMI is a positive predictor of serum 25(OH)D [2]. The main reason of the discrepancy may be relatively small sample size and different inclusion criteria. Therefore, the correlation between BMI and VD status in TB needs further study in larger populations.

A major limitation of this cross-sectional study is that it does not allow causal inference. Nevertheless, the evidence with respect to the possible predictors of serum 25(OH)D concentration and the prevalence of SVDD in active TB patients and the role of DM is worthy of further investigation. Since the epidemic of vitamin D deficiency in active TB is of great significance, we suggest that various strategies concerning the improvement of VD status such as more outdoor activity, higher consumption of fish, egg,

etc and fortified items, or use of VD supplements, should be given full consideration in this population.

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5 Is low serum 25-hydroxyvitamin D a possible link between pulmonary tuberculosis and type 2 diabetes?

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Abstract

Background and Objectives: Although vitamin D is implicated in the generation of anti-microbial peptide cathelicidin, which plays a key role against pulmonary tuberculosis (PTB), and may have an inverse association with the risk of type 2 diabetes (DM), its role in the co-existence of these two diseases (PTB-DM) is still uncertain. This study explored the association of vitamin D status with prevalent PTB, PTB-DM and DM.

Methods and Study Design: We randomly selected 130 PTB patients, 90 PTB-DM, 91 DM and 134 controls. Serum 25(OH)D was determined. A structured questionnaire and anthropometric measurements were administered.

Results: Serum 25(OH)D in PTB and PTB-DM were 12.2±2.2 ng/mL and 12.9±2.5 ng/mL, respectively, which were lower than those in DM and control groups.

Odds ratios of PTB and PTB-DM comparing extreme quartiles of 25(OH)D (lower than 8.6 ng/mL versus ≥26.6 ng/mL) were 3.26 and 2.27, respectively. These associations remained after adjustment for possible risk factors [OR (95% CI)=4.73 (2.04-10.9) and 2.50 (1.04-6.02), respectively]. A synergistic interaction was observed between low 25(OH)D and underweight in respect to prevalent PTB-DM [OR=24.6 vs 2.50 for lowest quartile of 25(OH) D and 4.59 for underweight].

Conclusions: Odds ratios of low serum 25(OH)D for PTB and PTB-DM were greater than 1.0, and were even much greater when combined with underweight. However, since the association of serum 25(OH)D with PTB was stronger than with PTB-DM, we could not draw the conclusion that vitamin D is a link between PTB and DM.

Introduction

The co-morbidity of pulmonary tuberculosis (PTB) and type 2 diabetes (DM) represents a double burden with significant public health implications[1]. With increasing global prevalence of DM that is anticipated to reach 552 million by 2030[2], and the continued high rates of tuberculosis in developing countries, the number of individuals with both diseases will increase markedly in the coming decades. Until now, the mechanisms that might underlie this association are still uncertain.

Vitamin D, which is mainly derived from endogenous synthesis after exposure of the skin to solar ultraviolet radiation, has been proven to have more functions in the body than the classical effects on calcium metabolism. Receptors for its active form, 1,25-dihydroxyvitaminD$_3$, are widely expressed in human cells, including pancreatic β-cells as well as numerous cell types of

the immune system such as monocytes and macrophages, dendritic cells, T cells, B cells, and natural killer cells[3; 4].

Epidemiologic studies have shown a higher incidence of TB disease in populations with diminished 25-hydroxyvitamin D [25(OH)D][5]. It is hypothesised that vitamin D is closely associated with the onset and treatment of active tuberculosis. Sufficient vitamin D can decrease the risk of infection with MTB and the progression of active tuberculosis from latent TB, and may decrease the duration and improve the treatment outcome [6; 7; 8; 9]. Vitamin D was also discovered to mediate the important innate antimicrobial immune response against MTB in vitro [10].

At the same time, the associations between vitamin D and DM have been reported recently. Cross-sectional studies showed that 25 (OH)D concentration was lower in individuals with DM and impaired glucose tolerance than in those with normal glucose tolerance [11]. A prospective study [12] in 1080 subjects of 5 year follow-up suggested that participants with 25(OH)D deficiency had an increased risk of DM, and the supplementation may be protective [13; 14]. Associations of 25(OH)D with insulin resistance and β cell function were reported by some authors [15], whereas others did not find an association [16].

It was supposed by Handel et al that Vitamin D may be the missing link between TB and DM [17]. However, there were hardly any related epidemiological reports. The current case control study was carried out to investigate whether lower serum 25(OH)D might be associated with higher prevalence of PTB, PTB-DM and DM, which might provide evidence for a role of vitamin D in the co-morbidity of these two diseases.

Methods

Study Population

The subjects were randomly selected from a previous large scale community based study of the prevalence of DM in active PTB patients and non-TB subjects in rural area in China [18]. The investigation study was registered in the Chinese Clinical Trial Registry (No. ChiCTR-OCC- 10000994, URL: http://www.chictr.org/cn/proj/show.aspx?proj=411).Briefly,newly-diagnosed PTB patients, 18 to 85 years of age, who registered for Directly Observed Treatment, Short Course (DOTS) were recruited consecutively from 7 TB clinics (Yishui TB clinic, Yinan TB clinic, Lanshan TB clinic, Cangshan TB clinic, Tancheng TB clinic, Feixian TB clinic and Pingyi TB clinic) in Linyi area (Linyi, China) from September 2010 to December 2012. PTB was diagnosed by chest radiography followed by sputum smear examination or sputum culture for those with a suspicious TB symptoms and shadow on chest X-ray. Diagnosis of diabetes was based on WHO criteria for the classification of glucose tolerance based on fasting plasma glucose (FPG). HIV-positive patients as well as subjects with type 1 diabetes, trauma in the last three months, cancer, sever cardiac, hepatic and kidney diseases were excluded. Cluster random sampling was used to recruit non-TB subjects from the same communities as TB cases, using the same exclusion criteria. Stratification was performed based on economic level (low/middle/high). Fasting blood samples were obtained from the participants for screening of DM. According to the DM screening results, the subjects were divided into four groups including PTB patients with DM (PTB-DM) and without DM (PTB), non-TB subjects with DM (DM) and without DM (NON). Finally, the study comprised 130 PTB, 90 PTB-DM, 91 DM and 134 NON.

Structured questionnaires were used by trained interviewers to collect information on demographic variables, medical history, medications, dietary and lifestyle habits. 24-hour dietary recall and food frequency questionnaire were used concerning the dietary habit together with the information of vitamin and mineral supplements. The Ethics Committee of Qingdao Disease Prevention and Control Centre approved the present study (No. 200904), and informed consent was obtained from each subject.

Laboratory analyses

Serum 25(OH)D was measured by using a radioimmunoassay (RIA) kit from DiaSorin Inc (DiaSorin, USA) in Beifang Institute of Biotechnology (Beijing,China). The sensitivity of the assay was 1.5 ng/mL. The inter assay variability was 10.5% and the intra assay variability was 8.2%. Lipid indexes including total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDLC) were estimated by enzymatic procedure.

Anthropometric measurements

Height and weight were measured by trained investigators using standard procedure. Body mass index (BMI, kg/m^2) was calculated by using the formula: BMI = weight (kg)/ height^2 (m²), and the cut-off value for Chinese population was used, as described previously [18].

Statistical analyses

Serum 25(OH)D exhibited a lognormal distribution, and data were therefore transformed (log10) before logistic regression analysis and Pearson's correlation analysis. Subjects were divided into quartiles based on their serum 25(OH)D. Multiple logistic regression analysis was used to evaluate the association(s) between serum 25(OH)D and PTB, PTB-DM or DM, with appropriate adjustment for covariates, including age [(years) (categorized in 3 units: <30, 30-49, ≥50], sex, BMI [(kg/m^2) (categorized as <18.5, 18.5-23.9,

≥24.0)], family history of DM and former smoking (smoking index, package per year multi- plied with smoking year was used and categorized in 4 units: 0, <15, 15-29, ≥30). The model fit was significant (χ^2=203.15, p<0.001) and the fit was good (Pearson χ^2=448, p=0.12). All probability values were derived from 2-tailed analyses, and those below 0.05 were considered to be of statistical significance. Analyses were performed with SPSS version 21.0 software (IBM SPSS Statistics 21).

Results

Characteristics of the study population

The general characteristics of the study population are displayed in Table 5-1. Patients with PTB-DM were older than PTB and NON (p<0.05). Male proportion was highest in PTB. And BMI in PTB and PTB-DM was lower than in the other two groups (p<0.05). There existed a difference in the lipid profile among these groups. Former smoking, evaluated by smoking index (SI) of package per day multiplied with smoking years, was more common among PTB cases and heavy smoking (SI ≥30) was more common in PTB-DM and PTB groups. Patients with PTB-DM and DM were more likely to have a family history of diabetes. Serum 25(OH)D concentrations were significantly lower in PTB and PTB-DM groups than in DM and controls (p<0.05).

Most of the subjects were peasants, with an educational level lower than college. Occupation, education level and alcohol drinking history were distributed equally in the four groups, as also were the seasons in which the blood samples were collected.

Table 5-1. Basic characteristics of the subjects ($\overline{x}$±SD)

	PTB	PTB-DM	DM	NON	p
N	130	90	91	134	
Age	45.6±18.7[*]	56.9±14.5[*,**]	55.8±13.8[**]	51.7±15.8	<0.05
Male, n(%)	102(77.3)	54(61.4)	55(61.1)	60(44.4)	<0.05
BMI	20.3±2.83[*]	20.9±3.32[*]	22.9±2.61	22.1±2.79	<0.05
SBP(mmHg)	120±12.7	119±14.0	124±13.3[**]	121±11.5	<0.05
DBP(mmHg)	75.7±8.14	78.0±6.60[**]	79.8±10.0[**]	79.5±6.85[**]	<0.05
Hypertension,n(%)	14(10.8)	10(11.1)	22(24.2)	20(14.9)	<0.05
FPG(mmol/L)	4.66±1.19	7.22±1.25	7.15±1.21	4.59±1.17	<0.05
TC(mmol/L)	4.11±1.17	5.03±1.32[**]	4.68±1.01[**]	4.78±1.27[**]	<0.05
TG(mmol/L)	0.95±0.49	1.23±0.67[*,**]	1.25±0.54[*,**]	0.85±0.58	<0.05
HDLC(mmol/L)	1.52±0.66[*]	1.67±0.59[*]	1.69±0.49[*]	1.99±0.73	<0.05
VitaminD(ng/mL)[†]	12.2±2.15[*]	12.9±2.51[*]	17.86±1.98[**]	16.69±2.02[**]	<0.05
SI					
0	75(59.1)	59(67.8)	70(80.5)	94(73.4)	<0.05
< 15	16(12.6)	2(2.3)	4(4.6)	18(14.1)	
15～	17(13.4)	6(6.9)	5(5.7)	12(9.4)	
≥ 30	19(15.0)	20(23.0)	8(9.2)	4(3.1)	
DM Family history	10(7.8)	19(21.2)	22(24.2)	6(4.5)	<0.05

Note: PTB: pulmonary tuberculosis patient without diabetes; PTB-DM: pulmonary tuberculosis patient with diabetes; DM: non-TB subjects with diabetes; NON: non-TB subjects without diabetes; BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; TG: triglyceride; HDLC: high density Lipoprotein Cholesterol; SI: smoking index.

[†]Log10 transformed, then back-transformed for presentation. *p<0.05 compared to NON group;**p<0.05 compared to PTB group.

Determinants of PTB-DM

After adjustment for potential confounders including sex, age, DM family history, BMI and smoking index, log serum 25(OH)D showed a protective association with PTB-DM (adjusted OR 0.37, 95% CI 0.15-0.92). Also,association was observed with PTB (adjusted OR 0.22, 95% CI 0.09-0.52). A clear association of serum log25(OH)D with DM was not observed (p>0.05) (see in Table 5-2).

Table 5-2. Multivariate logistic regression analysis of odds ratios on PTB, PTB-DM, and DM

	PTB	PTB-DM	DM
	OR(95%CI)	OR(95%CI)	OR(95%CI)
Men	2.76(1.46~5.19)*	1.60(0.80~3.22)	2.34(1.21~4.51) *
<30	1.00	1.00	1.00
30~	0.43(0.19~0.98)*	4.68(1.12~19.5)*	2.75(0.72~10.5)
≥ 50	0.36(0.16~0.78)*	7.64(1.90~30.8)*	7.69(2.09~28.3)*
DM family history	0.90(0.28~2.86)	6.62(2.21~19.8)*	7.91(2.73~22.9)*
BMI			
18.5~23.9	1.00	1.00	1.00
<18.5	1.88(0.87~4.09)*	4.73(2.06~10.9)*	0.41(0.11~1.55)
≥24	0.37(0.16~0.83)*	1.29(0.60~2.75)	2.09(1.06~4.12)*
SI			
0	1.00	1.00	1.00
<15	0.68(0.29~1.58)	0.12(0.02~0.57)*	0.28(0.08~0.90)*
15~	1.31(0.52~3.30)	0.35(0.11~1.16)*	0.30(0.09~0.99)*
≥ 30	3.91(1.15~13.3)*	4.20(1.20~14.7)*	1.26(0.33~4.84)
LogVD[†]	0.22(0.09~0.52)*	0.37(0.15~0.92)*	1.30(0.50~3.37)

PTB: pulmonary tuberculosis patient without diabetes; PTB-DM: pulmonary tuberculosis patient with diabetes; DM: non-TB subjects with diabetes; BMI: body mass index; SI: smoking index. [†]Log10 transformed.

Relationship between vitamin D concentrations and PTB-DM

The relationship between serum 25(OH)D and the presence of PTB, PTB-DM and DM is presented in Table 5-3. Subjects in the lowest quartile of 25(OH)D were about 3 times more likely to have PTB than those in the highest quartile (OR 3.26, 95%CI 1.56-6.82). This relationship was maintained after adjustment for other possible con- founders, including age, sex, body mass index, family history of DM and former smoking (adjusted OR 4.73, 95% CI 2.04-10.9). An decreased association was observed between 25(OH)D and PTB-DM. Subjects in the lowest quartile of 25(OH)D (<25th) were 2 times more likely to have PTB-DM than those in the highest quartile (OR 2.27, 95% CI 1.05-4.92) and the adjusted OR was2.50 (95% CI 1.04-6.02). There was no significant association between 25(OH)D and the prevalence of DM.

Table 5-3. Odds ratios for PTB, PTB-DM, DM by quartiles of serum 25(OH)D (ng/mL)

Vitamin D	≥26.62	15.40～26.61	8.58～15.39	≤8.57
N	111	112	111	111
PTB				
N (%)	23(17.7)	32(24.6)	30(23.1)	45(34.6)
Crude OR	1.00	1.32(0.65~2.67)	1.11(0.55~2.26)	3.26(1.56~6.82)[*]
Adjusted OR[†]	1.00	1.64(0.74~3.59)	1.35(0.61~2.97)	4.73(2.04~10.9)[*]
PTB-DM				
N (%)	22(24.4)	18 (20.0)	20(22.2)	30(33.3)
Crude OR	1.00	0.77(0.36~1.68)	0.78(0.36~1.65)	2.27(1.05~4.92)[*]
Adjusted OR[†]	1.00	0.97(0.42~2.27)	0.79(0.34~1.83)	2.50(1.04~6.02)[*]
DM				
N (%)	31(34.1)	25(27.5)	20(22.0)	15(16.5)
Crude OR	1.00	0.76(0.38~1.54)	0.55(0.27~1.13)	0.81(0.36~1.83)
Adjusted OR[†]	1.00	0.79(0.36~1.72)	0.58(0.26~1.29)	0.86(0.34~2.16)

PTB: pulmonary tuberculosis patient without diabetes; PTB-DM: pulmonary tuberculosis patient with diabetes; DM- non: TB subjects with diabetes.

[†]Adjusted for age (categorical), sex, BMI (categorical), family history of DM, SI (categorical).[*]p<0.05 compared to the highest quartile (≥26.62 ng/mL).

Synergistic association of 25(OH)D and BMI on the potential risk of PTB-DM

A synergistic association was observed when the lowest quartile of 25(OH)D and the underweight category (BMI<18.5) were considered together. After adjustment for the possible confounders, the odds ratio of having PTB in subjects with both characteristics was 14.1 (1.56-128). This association was enhanced with a nearly 2-fold increased odds ratio of 24.6 (95% CI 2.55-242) with prevalent PTB-DM compared with persons with highest quartile of 25(OH)D and normal weight, while the odds ratio was 2.50 (95% CI 1.04-6.02) for lowest quartile of 25(OH)D and 4.59 (95%CI 1.98-10.6) for underweight separately.

Discussion

Lower serum 25(OH)D was significantly associated with higher prevalence of PTB and PTB-DM after adjustment for confounders. A synergistic interaction was observed between underweight (BMI <18.5) and low 25(OH)D.

It has been indicated by several case-control studies and large scale longitudinal cohort studies that DM will increase the risk of active TB [19]. However, the link behind the association is not fully understood. Several studies have suggested that DM depresses the immune response through

effects on macrophage and lymphocyte function, which in turn facilitates active TB disease. Conversely, it is also possible that TB induces glucose intolerance and deteriorates glycemic control in subjects with DM [19; 20].

It has been indicated that vitamin D is associated with antimicrobial immune activity of human macrophages [21]. In vitro, the actions of monocytes and macrophages on Mycobacterium tuberculosis (MTB) are heavily dependent on vitamin D concentrations [13], and the antimicrobial peptide cathelicidin was induced by the increased expression of the vitamin D receptor and the vitamin D 1-hydroxylase genes in human macrophages, which may play a key role in killing of intracellular MTB [10]. Also, a growing body of evidence from observational studies suggests an association between low 25(OH)D and increasing DM risk [22] with impaired pancreatic β cell function being the possible mechanism [23].

The present study, therefore, compared the odds ratio of prevalent PTB, PTB-DM and DM in individuals with lower concentrations of serum 25(OH)D to that in individuals with higher concentrations, to check for evidence that vitamin D might be a link that explain part of the association between these two diseases.

We found a negative association between 25(OH)D and prevalent PTB, which was in accordance with other reports [5; 6; 24]. However, the odds ratio was clearly lower for the association between 25(OH) D and prevalent PTB-DM. Till now, the association of vitamin D and the prevalence of PTB-DM has not been reported. The lowered odds ratio compared with PTB alone indicates that vita- min D may not be a link between PTB and DM.

Given the association of BMI with 25(OH)D in PTB-DM, it might be that combined lower 25(OH)D and underweight reflects a heightened potential risk that is associated with or drives the progress of PTB-DM. Moreover, it might therefore be possible to use the combined information of these

factors to better estimate an individual's possible risk of having PTB-DM, and combined low 25(OH)D and underweight may be a possible link between PTB and DM. As we have discussed in our former study [20], the association for BMI in the setting of comorbid DM and TB is complex. While increasing the risk of DM, increased BMI is a protective factor against developing TB [25]. Weight loss due to poorly controlled DM and metabolic de-compensation takes away this protection, and would result in significant weight loss in patient with combined TB and DM [24]. Similarly, a study carried out in Tanzania[26] reported severe underweight (BMI <16 kg/m^2) among male TB patients was associated with DM. However, the number of subjects with both characteristics was relatively small (n=23), which may explain partly the wide confidence interval: 2.55-242. Therefore, the precise evaluation of the possible synergistic effect of low vitamin D level and underweight and its possible link of PTB and DM should be investigated in studies of larger sample size preferably prospective.

Other than what had been expected, low 25(OH)D were not significantly associated with the prevalence of DM. Similarly, a 5-year follow-up study found that low 25(OH)D status was not significantly associated with incident diabetes but it was indicated that low vitamin D status could be related to deterioration of glucose homeostasis [27]. However, Pittas et al reported that the relative risk of type 2 diabetes was 0.87 (95% CI 0.75-1.00; p for trend=0.04) comparing the highest with the lowest category of vitamin D intake from supplements [14]. Hitherto, strong epidemiological evidence of a link between vitamin D deficiency and increased risk of type 2 diabetes has been limited, since most studies were cross sectional [28]. Therefore, more longitudinal studies are needed to get a full insight into the association.

In conclusion, we observed an inverse correlation between circulating concentrations of vitamin D and the prevalence of PTB-DM, while we did not find any evidence that low vitamin D was a link between PTB and DM. It was

also indicated that low 25(OH)D and under- weight might cooperate in the co-occurrence of PTB and DM. The precise mechanisms underlying the relationship need further investigation.

Limitations

A major limitation of our study is that it may not allow causal inference. Vitamin D status might be a consequence of the disease status instead of a cause. Furthermore, despite adjustment for confounders, residual confounding cannot be completely ruled out. Most of the DM patients in this community based study were non-insulin dependent diabetes mellitus (NIDDM), the severity of DM with PTB and the role of vitamin D could not be analyzed. Nevertheless, the evidence with respect to the association of vitamin D with PTB and PTB-DM based on our study are worthy of further investigation.

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6 Poor Vitamin D Status in Active Pulmonary Tuberculosis Patients and its Correlation with Leptin and TNF-α

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Abstract

Vitamin D deficiency (VDD) is common in active tuberculosis (TB) patients and may be implicated in the etiology of the disease and in its clinical course. The aim of this study was to investigate the association between leptin, inflammatory markers and vitamin D status in TB patients, stratified for presence or absence of diabetes mellitus (DM). As part of a supplementation trial, at baseline we carried out a cross-sectional study. Two hundred ninety-nine active TB patients aged 18-65 years were recruited from Qingdao TB clinics from October 2015 to August 2016. Also, ninety-one normal controls undergoing routine medical examination were included from a general hospital in Qingdao. The information including socio-demographics, dietary intake and living habits was obtained from all the subjects by face-to-face interview using structured questionnaire. Serum concentrations of leptin and the inflammatory markers TNF-α, CRP and IL-6 were compared between TB patients with and without VDD and severe VDD (SVDD). Pearson's correlation was used to analyze the association between

the concentrations of TNF-α, leptin and 25-hudroxyvitamin D [25(OH)D]. A significantly higher prevalence of VDD and SVDD was observed in TB patients compared with normal controls (93.0% vs 70.3%, 65.9% vs 3.3% respectively). Concentration of leptin was significantly lower, while TNF-α higher in TB patients with SVDD compared to those without (P<0.05). After adjustment for confounders such as BMI, age and gender, leptin concentration was positively associated with 25(OH)D (r= 0.210, P=0.002) with similar correlation in TB patients with DM (r=0.240, P=0.020). A negative association between serum TNF-α and 25(OH)D was observed (r=-0.197, P=0.003), which was significant only in the subgroup without DM (r=-0.304, P=0.001). In contrast, no correlation between both leptin and TNF-α with serum 25(OH)D was found in normal controls. Our findings indicate that a higher vitamin D status in TB patients may be related to higher immune activity and less serious tissue damage, and that this relation is different according to presence or absence of DM co-morbidity.

Key words: serum 25(OH) D; tuberculosis; leptin; TNF-α; diabetes
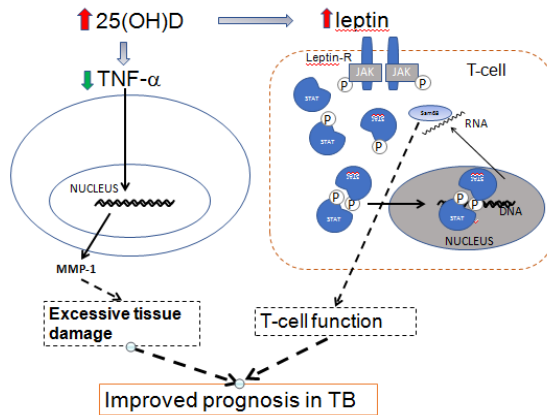
→ Promote; Leptin-R,leptin receptor; TNF, tumor necrosis factor; CRP, C-reactive protein; TB,tuberculosis; JAK,Janus kinase; STAT, signal transducer and activator of transcription; MMP, matrix metalloproteinase; Ⓟ, phosphorylate.

The present study in active TB patients found that serum TNF-α was independently inversely correlated with 25(OH)D, while leptin levels showed a positive correlation with VD. TNF-α has been reported to be closely related to excessive tissue damage in TB, while optimal leptin levels were related to normal T-cell function in the body. Therefore, we supposed that the modification of certain cytokines such as TNF-α and leptin by vitamin D may be one of the underlying mechanisms of the immune-modulatory effects of vitamin D in TB patients.

Introduction

Tuberculosis (TB) continues to be a major public health problem. According to the global TB report (WHO), the epidemic was larger than previously estimated. There were a total of 10.4 million new TB cases worldwide in 2016, with China still ranking high among the epidemic countries [1]. China is also facing a rapidly increasing incidence of diabetes mellitus (DM) in the past ten years [2]. DM has been reported to triple the risk of active TB[3]and to result in poor response to anti-TB treatment [4]. However, the underlying mechanisms are still uncertain.

In recent years, the non-skeletal function of vitamin D (VD) has aroused intense interest. VD was discovered to stimulate innate immunity during Mycobacterium tuberculosis (*Mtb*) infection resulting in control of *Mtb* proliferation inside macrophages [5; 6]. It is also involved in the regulation of host cytotoxic T lymphocyte responses [7] and the differentiation of naive T cells to regulatory T cells, indicating a possible role in adaptive immunity during infections.

VD deficiency (VDD) is a global health problem [8]. High prevalence of VDD has been documented in Europe, Southeast Asia [9], Middle East and South

America [8], even in some areas with very sunny climates, such as Saudi Arabia and India [10; 11]. High prevalence of VDD was reported in TB patients. A meta-analysis combining seven epidemiological studies showed a modest to strong association of VDD with tuberculosis risk, reporting serum vitamin D levels are 0.68 SD lower in people with tuberculosis compared to controls [12]. Although still lacking solid evidence, VD supplementation was reported to accelerate sputum smear conversion and the resolution of inflammatory responses during tuberculosis treatment [13].

Cytokines play an important role as mediator in the interaction between immune cells [14]. The production of pro-inflammatory cytokines such as TNF-α indicates an important immunity response against *Mtb in the host* [15]. However, exacerbated inflammatory responses may lead to poor bacterial control and development of TB in the host [16]. It was reported that a high level of TNF-α during the treatment may lead to deleterious effects in the tissues, while anti-TNF-α antibody during the treatment resulted in augmented bacterial clearance and attenuated lung pathology, which indicates TNF-α level may be a marker of exaggerated inflammation during TB course [17]. VDD was reported to be correlated with increased production of pro-inflammatory cytokines [18], which indicates the possible mechanism of the role of VD in TB. Although a potential role for VD in modulation of the inflammatory response in TB patients has been suggested [13], most of the available evidence is provided by in vitro studies [19; 20], and direct proof of the correlation of inflammatory levels with VD status in active TB patients is still lacking.

Leptin, a "satiety" hormone predominantly secreted by adipose cells to help to regulate energy balance, is also involved in both innate immune and adaptive immunity [21; 22; 23]. Falling leptin concentrations appeared to be responsible for reduced T-cell function during starvation [24]. A recent meta-analysis of twelve case-control studies found serum leptin levels of

healthy controls were markedly higher than those of PTB patients [25], and after treatment of TB plasma leptin levels were observed to increase [26]. However, the correlation between leptin and VD in TB patients has not been reported until now.

It is now well established that chronic inflammation, indicated by modestly increased levels of cytokines, contributes to the development and progression of DM. Impaired immune function in diabetic patients, especially with respect to macrophage and T lymphocyte functions, play a key role in the defence against M. tuberculosis[27; 28; 29], contributing significantly to higher risk of TB in DM patients. It was reported that tuberculosis patients with diabetes had a different profile of circulating levels of cytokines compared with those without diabetes[30].

We hypothesize that there may exist certain correlations between inflammatory cytokines, leptin and VD status, which may underlie the mechanism of the immune-modulatory effects of VD in TB, and this correlation may differ depending on the presence or absence of comorbid diabetes. Therefore, we carried out this cross-sectional study in active TB patients with and without DM. At the same time, a normal control group was included to compare the possible correlation between inflammatory cytokines, leptin and VD status.

Methods

Design and subjects

In this cross-sectional study, 299 active TB patients (178 TB without DM and 121 TB with DM) were recruited from Qingdao TB clinics from October 2015

to August 2016. Also, 91 normal controls undergoing routine medical examination were included from a general hospital in Qingdao. The diagnosis of TB was made according to standard clinical criteria in which clinical manifestations, sputum smear microscopy and chest radiography were the central components. Diagnosed TB patients registered for Directly Observed Treatment, Short Course (DOTS) before the start of TB treatment, aged 18-65 years were recruited. The diagnosis of DM was based on self-report by patients who had already taken anti-diabetic medicines, or on the results of fasting plasma glucose (FPG) at the routine admission biochemistry test according to WHO criteria (1990) for the classification of glucose tolerance. Volunteers were excluded if they were pregnant or HIV-positive, had cancer, severe cardiac, hepatic and kidney diseases or trauma in the last three months, took VD supplements in the former 6 months, had a current or previous medical condition or took medication affecting immune function or affecting VD status in the body.

This study was carried out in accordance with requirements documented in the Declaration of Helsinki. Ethical approval for this study was received from the Ethics Committee of Qingdao Disease Prevention and Control Centre (Qingdao, China), and informed consent was obtained from each subject. The study was registered in the Chinese Clinical Trial Registry (No. ChiCTR-IPR-15006395).

Questionnaires and anthropometrical measurements

Structured questionnaires were administered by face-to-face interview to all subjects to collect information of socio-demographics, previous health conditions or diseases and family disease history, as well as lifestyle habits including smoking, alcohol drinking and outdoor activity level.

Height and weight were measured without shoes and heavy clothing by trained investigators and body mass index (BMI) was calculated as body

weight divided by height squared (kg/m^2). The cut-off value for Chinese population [31] was used for the classification of underweight (<18.5), normal weight (18.5-23.9), overweight and obesity (≥24).

Biochemical detection and laboratory analyses

Blood samples were drawn from each subject between 6:30 am and 9:00 am after fasting for at least 12 h and an overnight rest. Venous blood was collected into vacutainer tubes and allowed to clot at room temperature for 30 minutes. The coagulated blood was centrifuged to collect the serum. Glucose levels were measured by glucose oxidase method and lipid indexes including serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC) were determined by enzymatic procedure. All the analysis were performed by an automatic biochemical analyzer. The rest serum was aliquoted into sterile microcentrifuge tubes, and stored at -80°C until measurement of 25(OH) D, leptin and inflammatory indexes of TNF-α, IL-6 and CRP.

Serum 25(OH)D concentrations were measured as described before [32]. Briefly, ^{125}I radioimmunoassay (RIA) kit from DiaSorin Inc (Stillwater, USA) was used. The sensitivity of the assay was 1.5 ng/mL. The inter-assay variability was 10.5% and the intra-assay variability was 8.2%.

Leptin levels were determined by the method of radioimmunoassay (RIA, XH6080). The sensitivity of the assay was <0.4ng/mL. The inter-assay variability was <10% and the intraassay variability was <15%, and the average recovery was 93%-99%.TNF-α and IL-6 levels were also measured by ^{125}I radioimmunoassay kit. For TNF-α, the sensitivity of the assay was 6 fmol/mL; the inter-assay variability was <10% and the intra-assay variability was <15%.For IL-6, the sensitivity of the assay was 50pg/mL; the inter-assay

variability was <7% and the intra-assay variability was <15%.Serum levels of CRP were measured by immuno-turbidimetric assay.

Definition of VDD and SVDD

The current widely accepted vitamin D nutritional status evaluation criteria by the Institute of Medicine was used to define vitamin D deficiency (VDD) and severe vitamin D deficiency (SVDD) [33]. Serum 25(OH)D <20ng/ml (50 nmol/L) was defined as VDD, and Serum 25(OH)D <10ng/ml(25nmol/L) was defined as SVDD.

Statistical analyses

Characteristics of TB patients and normal controls were presented, also the results of comparison between TB patients with and without SVDD. Mean and standard deviation for continuous variables with normal distribution and proportions for categorical variables were reported. Median and inter-quartile range were used for the description if the continuous data that were not normally distributed based on the results of Kolmogorov–Smirnov test. If the data were normally distributed after log10 or ln transfer, anti-log10 or anti-ln values were presented.

Independent-samples T test was used to determine the differences between the groups; for data not normally distributed or of unequal variance, rank sum test was performed. Spearman's correlation analysis was used to determine the relationships between serum leptin, TNF-α and 25(OH)D, and these data were log10- or ln-transformed into normal distribution data for correlation analysis. Also, partial correlation analysis was used to adjust for the influence of BMI, age and gender. All statistics were performed using SPSS version 21.0 software (IBM SPSS Statistics 21). A p-value of <0.05 was considered statistically significant.

Results

General characteristics of the participants

General characteristics of patients and controls are shown in Table 6-1. No significant difference between age and gender was observed between the two groups. TB patients had lower total protein (g/L) (66.28±7.01 vs74.16±3.84), albumin (g/L) (39.12±5.80 vs 45.97±2.44), hemoglobin (g/L) (126.64±18.99 vs144.73±15.94), lymphocyte count (0.51±0.61vs 2.05±0.60) and lymphocyte percentage (%) (24.36±11.31 vs 35.57±7.65) compared with controls, indicating decreased nutritional status in TB patients. Serum 25(OH)D (ng/mL) in TB patients was significantly lower than normal controls (6.50±0.22 vs 17.21±0.14), which was nearly 40% of the latter. The prevalence of VDD was higher (93.0% vs 70.3%), and of SVDD much higher (65.9% vs 3.3%) than in controls.

Table 6-1. General characteristics of the participants (Mean± SD)

	TB patients	Control	P-value
N	299	91	
Age	45.14±16.45	47.45±9.24	0.203
Male, n(%)	219(73.2)	63(69.2)	0.504
SBP	123.60±17.08	127.23±17.49	0.083
DBP	76.11±10.75	79.06±12.27	0.026
BMI	22.03±3.41	22.78±2.98	0.068
FPG(mmol/L)^	5.42,3.58	4.86,0.55	0.000
TC(mmol/L)	4.19±1.12	4.76±0.56	0.000
TG(mmol/L)*	1.03±0.18	0.88±0.15	0.019

HDL-C(mmol/L)*	1.19±0.14	1.44±0.13	0.013
LDL-C(mmol/L)^	2.42,1.03	2.71,0.73	0.207
TP(g/L)	66.28±7.01	74.16±3.84	0.000
ALB(g/L)	39.12±5.80	45.97±2.44	0.000
ALT(U/L)*	15.59±2.35	16.94±1.58	0.382
AST(U/L)^	15.00,11.00	20.00,7.00	0.000
GGT(U/L)*	32.80±2.07	1.99±1.61	0.000
BUN(mmol/L)	4.73±2.69	5.08±1.23	0.242
CRE(mmol/L)	57.31±35.79	62.60±13.05	0.168
Hb(g/L)	126.64±18.99	144.73±15.94	0.000
WBC count*	6.52±1.44	5.66±1.26	0.001
NEUT count*	4.32±1.65	3.10±1.36	0.000
NEUT%	67.92±12.65	55.42±8.46	0.000
LYM count	0.51±0.61	2.05±0.60	0.000
LYM%	24.36±11.31	35.57±7.65	0.000
MONO count*	2.95±1.79	3.40±1.36	0.026
MONO%*	4.50±1.93	5.60±1.28	0.000
25(OH)D(ng/mL)†	6.50±0.22	17.21±0.14	0.000
<20ng/mL, n(%)	278(93.0)	64(70.3)	0.000
<10ng/mL, n(%)	197(65.9)	3(3.3)	0.000

^Skewed distribution in the data, median and inter-quartile range were used for presentation and Mann-Whitney U test was used to test the difference. *log transferred and 10x for presentation, † $\log\times10$ transferred, and 10^x for presentation.

TB, tuberculosis; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body Mass Index; FPG, Fasting Plasma Glucose; TC, Total Cholesterol; TG, triglyceride; HDLC, High-density Lipoprotein Cholesterol; LDLC, Low-density Lipoprotein Cholesterol; TP, total protein; WBC, white blood cell; NEUT, neutrophils; MONO, Monocyte; LYM, Lymphocyte; 25(OH)D, 25-hydroxyvitamin D; VD, vitamin D; VDD, vitamin D deficiency;

SVDD, severe vitamin D deficiency; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

Difference according to presence or absence of SVDD and DM

As shown in Table 6-2, 299 TB patients were classified into two groups based on the cut-off value of SVDD (serum 25(OH)D<10 ng/mL). Log normal distribution of the data was found for serum 25(OH)D, Leptin, TNF-α, IL-6 and CRP. Leptin (ng/mL) concentration in SVDD group was significantly lower than in non-SVDD group (2.70±0.22 vs 3.70±0.19, P=0.0001), while TNF-α (fmol/mL) concentration was higher in SVDD group (12.47±1.89 vs 9.68±2.11, P=0.004). No obvious differences were observed in IL-6 and CRP levels.

When stratified by the presence of DM, 121 TB patients with DM (TB-DM) and 178 without DM (TB-nonDM) were identified. Compared with TB-nonDM, TB-DM had higher levels of serum 25(OH)D (ng/mL) (8.07±0.26 vs 5.61±0.23, p<0.001). Significant difference of TNF-α (fmol/mL) and CRP (mg/L) existed between them (10.07±2.02vs12.53±1.94, p=0.009 and 52.84±3.69 vs 16.52±4.80, p=0.000). No significant difference in leptin and IL-6 was observed between the two groups.

Table 6-2. Characteristics of TB patients by presence of SVDD（Mean± SD)

	SVDD	Non- SVDD	P-value
N	197	102	
Age	44.79±16.75	45.83±15.92	0.605
Male, n(%)	144 (73.1)	75 (73.5)	0.936
SBP	124.16±16.87	122.47±17.53	0.424
DBP	76.05±10.80	76.24±10.69	0.882
BMI	22.03±3.29	22.03±3.60	0.997
<18.5, n(%)	21(14.5)	14(14.6)	
18.5~23.9, n(%)	85(58.6)	59(61.5)	0.873
≥24, n(%)	39(26.9)	23(24.0)	
FPG(mmol/L)^	5.32,3.36	5.84,4.10	0.139
TC(mmol/L)	4.17±1.12	4.21±1.13	0.812
TG(mmol/L)†	0.97±0.17	1.14±0.18	0.028
HDL-C(mmol/L)†	1.17±0.14	1.24±0.14	0.184
LDL-C(mmol/L)^	3.29,0.94	2.49,1.09	0.510
Hemoglobin(g/L)	125.66±19.13	128.56±18.67	0.219
TP(g/L)	66.37±6.46	66.10±7.98	0.756
Albumin(g/L)	38.89±5.49	39.55±6.36	0.357
LYM count	1.50±0.58	1.51±0.65	0.950
LYM%	24.89±11.20	23.28±11.52	0.269
WBC count*	6.28±1.45	6.99±1.40	0.016
NEUT count	4.69±2.80	5.37±2.84	0.063
NEUT%	66.93±13.19	69.92±11.28	0.065
MONO count†	0.30±0.18	0.27±0.17	0.153
MONO%*	4.68±1.94	4.15±1.89	0.166
25(OH)D†(ng/mL)†	4.08±0.17	15.92±0.14	0.000
Leptin(ng/ml)$	2.70±0.22	3.70±0.19	0.001
TNF-α(fmol/mL)*	12.47±1.89	9.68±2.11	0.004

IL-6(pg/ml)*	85.50±2.68	90.78±2.06	0.593
CRP(mg/L)*	27.10±4.72	27.54±5.06	0.945
Outdoor activity			
≥ 2hrs/d,n(%)	60(40.3)	31(32.3)	0.207
< 2hrs/d,n(%)	89(59.7)	65(67.7)	
Smoking history, n(%)	48(24.4)	38(37.3)	0.020
Alcohol history, n(%)	75(38.1)	49(48.0)	0.097

† log×10 transferred,*log transferred, $log×10 transferred.^Skewed distribution in the data, median and inter- quartile range were used for presentation and Mann-Whitney U test was used to test the difference

TB, tuberculosis; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body Mass Index; FPG, Fasting Plasma Glucose; TC, Total Cholesterol; TG, triglyceride; HDLC, High-density Lipoprotein Cholesterol; LDLC, Low-density Lipoprotein Cholesterol; TP, total protein; WBC, white blood cell; NEUT, neutrophils; MONO, Monocyte; LYM, Lymphocyte; 25(OH)D, 25-hydroxyvitamin D; SVDD, severe vitamin D deficiency; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

Relationship between serum leptin and 25(OH)D levels in active TB patients

Leptin concentrations were significantly positively correlated with serum 25(OH)D in TB patients (r=0.246, P<0.001), which persisted after adjustment for BMI, age and gender (r=0.210, P=0.002). Stratified analysis showed that serum leptin concentrations were both positively correlated with 25(OH)D in TB patients with and without DM (r=0.240, r=0.182, respectively). R^2 -value in TB-DM was 0.104, which indicated 10.4% variation of serum leptin concentration could be explained by serum 25(OH)D. No significant correlation was observed between serum leptin and 25(OH)D in normal controls. (Figure6-1)
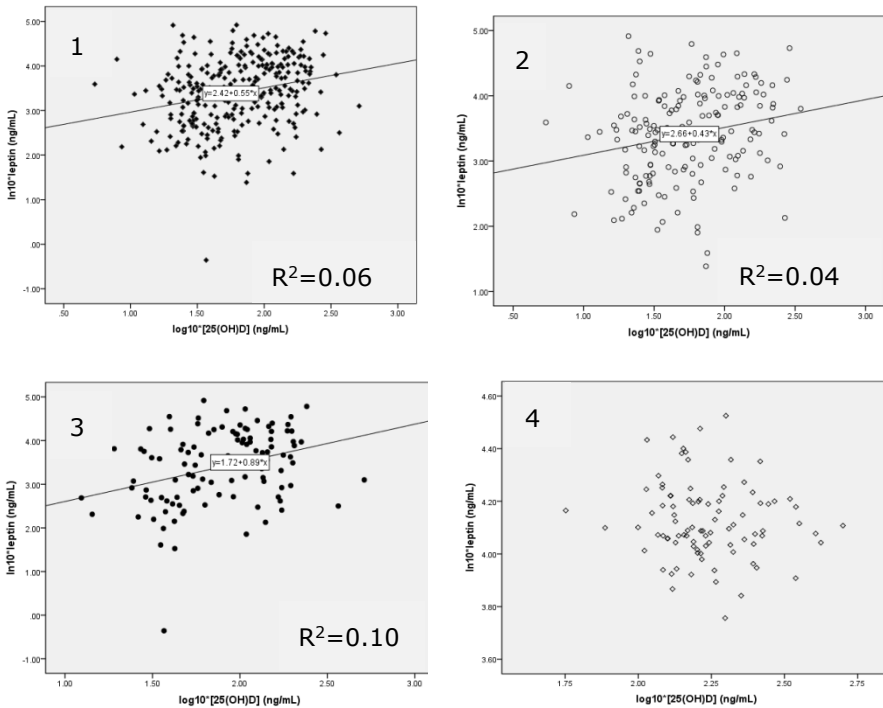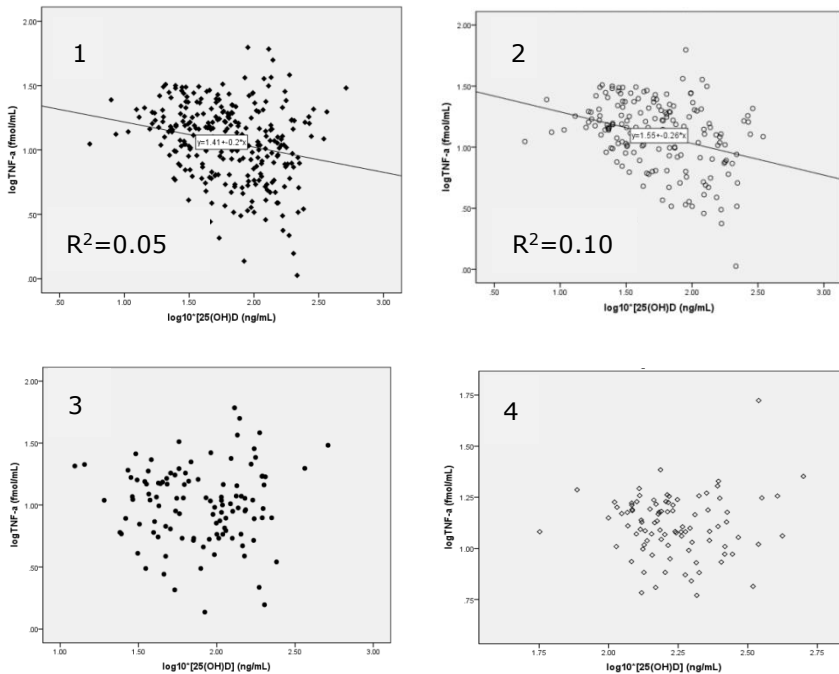
Figure 6-1. Correlation between serum leptin and 25(OH)D concentrations*

1, all TB; 2, TB-nonDM; 3, TB-DM; 4: normal control.*25(OH)D was log10* transformed, and leptin was ln10* transformed.

Relationship between serum TNF-α and 25(OH)D levels in active TB patients

A negative correlation between TNF-α and serum 25(OH) in active TB patients was observed (r=-0.226, P <0.001), which persisted after adjustment for BMI, age and gender (r=-0.197, P=0.003). Further stratified analysis by presence of DM showed different profiles. The correlation was stronger in TB patients without DM (r=-0.304, P = 0.001), and R^2 value was 0.104 which indicating that serum 25(OH)D status explained 10.4% of the variation in TNF-α concentrations. However, no such association was observed in TB-DM patients. The analysis of the correlation of TNF-α and 25(OH)D in normal controls gave no significant result. (Figure6-2)

Figure 6-2. Correlation between serum TNF-α with 25(OH)D concentrations[*]

1, all TB; 2, TB-non DM; 3, TB-DM; 4: normal control; *25(OH)D was log10* transformed, and TNF-α was log transformed.

Discussion

The present study demonstrates poor vitamin D (VD) status and a positive association between leptin and VD status in active TB patients, while the pro-inflammatory marker TNF-α was inversely correlated with VD. Furthermore, these correlations showed a different profile depending on presence or absence of comorbid DM, especially for TNF-α.

In this observational study, the participants were recruited from a city level hospital in Qingdao, located in Shandong province in Eastern China. This hospital has a catchment area of about 9 million people, and the average

number of hospitalized patients with tuberculosis was about two thousand cases per year. A total of 299 active tuberculosis patients were randomly recruited, together with 91 normal controls randomly selected from persons undergoing physical check-up in a general hospital in the same city. No significant difference of age and gender existed between the patients and controls, which supports comparability.

The most widely accepted criteria[33] were used to characterize the participants as VDD and SVDD in our study, i.e. cut off's for serum 25(OH)D of 20ng/mL and 10ng/mL, respectively. Serum leptin and TNF-α levels were highly correlated with BMI[34], also age, gender may explain variance in their levels in the body[35; 36]. These potential confounders were considered in the present study. Partial correlation analysis was used to adjust the possible confounding effect of these variables.

We observed a high prevalence of VDD (93.0%) and SVDD (65.9%) in active tuberculosis patients, which was significantly lower than in normal controls (70.3% and 3.3%, respectively).Together with our previously reported study in a partly overlapping population[37], we might draw the conclusion that the VD status in active TB patients is very poor, especially in hospitalized patients.

Serum leptin levels were found to be positively correlated with 25(OH)D concentrations in TB patients in the present study, which was in accordance with the result of a cross-sectional study in a cohort of 259 Saudi normal adults [38]. Leptin was reported to activate T lymphocytes via activating JAK-STAT signal pathway [39], together with the modification of Th1/Th2 balance, indicating its positive correlation with immune activity in the body. Low leptin levels may be a possible mediator of the association between severe weight loss and the suppressed cellular immunity[39; 40] in TB patients. Different to our result, an inverse association was claimed between leptin

and 25(OH)D concentrations in a recent meta-analysis of 14 cross-sectional studies [41]. However, most of the subjects included in this study were severely obese subjects, healthy persons or hemodialyzed patients, while no TB patients were included. The analysis stratified by DM showed similar results, while the correlation was somehow stronger in TB-DM patients. The possible reason may be that vitamin D is correlated with increased risk both of TB and DM [42; 43], which may have resulted in a closer link between VD and leptin in patients with the combined disease. To our knowledge, this is the first time to report a positive relationship between leptin concentrations and VD status in active TB patients, and our result indicates that a possible favorable role of VD may be mediated by up-regulating leptin level.

Serum TNF-α concentrations was inversely related to 25(OH)D in TB-nonDM patients, and about 10% of the variation in TNF-α concentrations was explained by VD. TNF-α, a pro-inflammatory cytokine secreted by macrophages, monocytes, T-cells and adipocytes, plays an important role in anti-TB immunity with a complex manifestation. Changes of TNF-α may be closely related to the progress of TB. A recent study claimed that TNF-α was correlated with TB severity, suggesting that it may be a marker of severity[44], and even a slight drop in circulating TNF-α may have clinical significance, indicating protection against excessive tissue damage at the site of infection [45].Until now, a few in vitro studies about the correlations between TNF-α and VD in TB are available.1,25-dihydroxyvitamin D (1,25(OH)D) stimulation led to elevated levels of TNF-α in human monocyte-derived macrophages from TB patients[46]. However, 1,25(OH)D was also reported to significantly suppress most *Mtb* antigen induced pro-inflammatory cytokines including TNF-α. Notably, our stratified analysis showed that this inverse correlation did not exist in TB-DM patients. As we found in this study, co-existing DM presented with a different TNF-α level in TB patients. Similarly, increased

inflammatory status was reported in TB-DM patients compared with TB[47]. This may partially explain the different correlation between VD and TNF-α in TB-DM patients from those with only TB. Our result indicates that the down-regulation of TNF-α by vitamin D may contribute to its effects in TB patients.

The main strength of this study is that it provided renewed evidence on the correlation of inflammatory indexes and leptin with VD in TB patients in an in vivo situation. This may add valuable clues of the possible mechanism of the effect of VD in active TB patients. Also, the modification of comorbid DM was analyzed and a different profile of the associations was observed, especially with respect to TNF-α. In the setting of double burden of TB and DM in developing countries in recent years, our study provides important data for the understanding of the characteristics of the diseases. Of course, the study has its limitations. We cannot make a convincing causal interpretation due to the cross-sectional design. In addition, despite adjustment for possible confounders in the data analysis, residual confounding cannot be completely ruled out. Nevertheless, the evidence with respect to the correlation of serum VD status with leptin and inflammatory biomarkers in active TB patients, and the heterogeneity observed in patients with and without DM is worthy of further investigation.

In conclusion, this study provides evidence that TNF-α in active TB patients is independently inversely correlated with circulating 25(OH)D concentration, while serum leptin levels show a positive correlation. This indicates that adequate VD status may lead to improved immune activity and less severe disease in TB. The underlying molecular mechanisms need further research.

Acknowledgements

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7 Vitamin D treatment in pulmonary tuberculosis patients with and without diabetes mellitus type 2: an 8-weeks cluster randomized controlled trial in China

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Abstract

Diabetes results in the presentation of more symptoms, more relapses, treatment failures and deaths during anti-tuberculosis therapy in patients with combined diseases. Therefore, new treatment strategies are urgently needed. Vitamin D has been shown to have beneficial effects towards clinical recovery such as sputum culture conversion. Therefore, we carried out this intervention study with the main purpose to explore the possible effects of vitamin D on treatment outcomes. A cluster randomized trial of vitamin D supplementation added to pharmacotherapy in tuberculosis patients with and without diabetes was carried out in a city-level chest hospital. Three hundred and fifty eight newly diagnosed pulmonary tuberculosis patients aged ≥18 y were included in this study, with 174 cases in VD3 supplementation group (800IU/d) and 184 in control group. The supplementation of vitamin D3 in patients with combined diseases showed

significant effects on the improvement of TB manifestation at 4th, 6th, 8th week, adjusted for confounders as age, gender. However, no similar effects of vitamin D were found in patients without diabetes. Our results provide an important clue of the role of vitamin D in the adjunctive treatment in patients with combined tuberculosis and diabetes, which may indicate a new perspective for treatment. However, the limitations of the trial need to be considered when making causal inference and more confirmative trials of the effect of vitamin D supplementation are needed.

Key words: vitamin D3; tuberculosis; diabetes; TBscore; supplementation

Introduction

Tuberculosis is a major global public health problem, with an estimated 9 million incident cases worldwide each year, mainly in low and middle income countries such as India, Indonesia, and China, which rank the top 3 of world tuberculosis burden in 2016 as WHO reported [1]. Over the last decades, diabetes mellitus has risen worldwide from 4.7% in 1980 to 8.5% in 2014 [2].Currently, 80% of global diabetes mellitus burden is in low and middle countries. Six of them (India, China, Brazil, Indonesia, Pakistan and The Russian Federation) also have a high tuberculosis burden[1]. In China, the prevalence of diabetes has increased especially rapidly over the last 10 years [3]. The International Diabetes Foundation Diabetes Atlas estimated that in 2017 the number of persons with diabetes in China was 114 million, representing nearly a quarter of cases worldwide. Diabetes triples the risk of active tuberculosis [4], and also results in the presentation of more symptoms, more relapses, treatment failures and deaths[5; 6], especially among those with poorly controlled diabetes [7]. According to a pooled analysis of four studies, diabetes was associated with nearly 5 times higher risk of death during tuberculosis treatment, adjusted for age and other potential

confounders[5; 8]. Therefore, new treatment strategies given the double burden of tuberculosis and diabetes are urgently needed.

Vitamin D may have an important role in host immune defense against Mycobacterium tuberculosis (*Mtb*) [9]. Vitamin D deficiency is common in active tuberculosis, and a meta-analysis in 2008 combining seven observational studies have found evidence of a moderate to strong association between vitamin D deficiency and active tuberculosis[10]. Several prospective longitudinal studies further claimed an association between vitamin D deficiency and higher risk of active tuberculosis [11; 12]. Accordingly, we found a high prevalence of vitamin D deficiency in tuberculosis patients, regardless of the presence of diabetes [13]. Also, Vitamin D deficiency has been associated with the severity and relapse rate of tuberculosis disease. Moreover, vitamin D deficiency has been reported to be related to impaired insulin secretion and sensitivity, and is associated with risk of diabetes[14; 15; 16]. Epidemiological evidence for an inverse association between serum vitamin D levels and the risk of type 2 diabetes is abundant [17]. A cross sectional study in overweight and pre-diabetic populations in China reported 83. 3 % vitamin D deficiency, and vitamin D status was inversely associated with insulin resistance [17]. A recent cohort study in Asia found that compared to individuals with vitamin D concentrations ≥ 20 ng/mL, those with concentrations of 10-19.9 ng/mL and of <10 ng/mL have a 2.06 and 3.23 fold increased risk of developing type 2 diabetes, respectively[16].

Indeed, vitamin D was used for treatment of tuberculosis in the pre-antibiotic era. It has been reported that addition of vitamin D3 to standard therapy had beneficial effects towards clinical recovery including sputum culture conversion [18], weight gain, chest radiographic findings [19] and resolution of inflammatory responses that are associated with increased

risk of mortality [20]. Recently, it was reported that the effect of vitamin D on tuberculosis therapy was modified by SNPs in genes encoding the vitamin D receptor (VDR) and 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) [21]. A possible mechanism of vitamin D–mediated effect on tuberculosis treatment efficacy is that 25-dihydroxyvitamin D induces anti-mycobacterial activity in macrophages in vitro, up-regulating protective innate host responses, and triggering antimicrobial peptides such as cathelicidin [9]. However, there is still controversy about the efficacy of adjunctive use of vitamin D on tuberculosis at present. Adding vitamin D to a background of standard first-line anti-tuberculosis drugs did not find a significant effect of on the rate of sputum *Mtb* clearance [22; 23; 24].

We report findings from a hospital-based trial testing the effect of vitamin D supplementation in tuberculosis patients with and without diabetes in China. We aimed to test whether vitamin D supplementation could improve clinical response to treatment in newly diagnosed pulmonary tuberculosis and accelerate sputum clearance of *Mtb*. Further, we checked whether the outcome in tuberculosis patients with concurrent diabetes might show a different profile.

Methods

Study setting

The trial was implemented in a city level specialized tuberculosis hospital located in Qingdao, Shandong province of China. This hospital has a catchment area of about 9 million people, and the average number of hospitalized patients with tuberculosis was about two thousand cases per year.

Study design and assignment of the participants

We conducted an intervention study, with an external control group from the same hospital. Diagnosed tuberculosis patients were assigned, in order of coming in, to one of the four clinical departments in the hospital in turn according to their visiting sequence. In all departments the same standard treatment procedure was used. One department was chosen randomly as the vitamin D intervention arm, and all consenting patients in this section received conventional anti-tuberculosis treatment plus oral vitamin D3 supplementation (800IU/d) for 8 consecutive weeks. One of the other departments was chosen as a control department, where patients received standard therapy. Physical examination including weight, height, face-to-face questionnaire was the same in both departments.

Study subjects

Pulmonary tuberculosis patients with and without diabetes were recruited between Jan 2000 and Dec 2012 from Qingdao tuberculosis hospital (Qingdao, China). Medical records of consecutive patients with diagnosed pulmonary tuberculosis disease were screened for eligibility. Potential study subjects were identified after diagnosis with the use of standard methods including of compatible signs and symptoms, and a chest radiograph.

Inclusion criteria were as follows: 1) age $\geq$ 18 y; 2) newly diagnosed pulmonary tuberculosis disease; 3) <7d of anti-tuberculosis drug therapy before entry. Exclusion criteria were as follows: 1) current extrapulmonary tuberculosis; 2) retreatment patients; pregnant or lactating woman; 3) HIV positive; 4) a history of nephrolithiasis, hyperparathyroidism, organ transplant, hepatic cirrhosis, or cancer in the past 5 y; 5) a baseline plasma calcium concentration >2.6 mmol/L, creatinine concentration >250 mmol/L, or aspartate aminotransferase concentrations that was >3 times the upper

limit of the normal range; 6) corticosteroid use in the past 30 d; 7) current use of immunosuppressive drugs.

The Ethics Committee of Qingdao Disease Prevention and Control Centre approved the present study, and consent was obtained from all subjects. This investigation research was registered in the Chinese Clinical Trial Registry (No. ChiCTR-IPR-15006395).

TBscore for evaluation of the severity of tuberculosis related clinical signs and symptoms

TBscore is a tool aiming to assess changes in the clinical status in patients with tuberculosis. It is based on points assigned to signs and symptoms, including cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, BMI<18 and BMI<16, as we previously stated [13].

The severity of tuberculosis clinical signs and symptoms was categorized based on the distribution of TBscore in the present study: class 1(mild, TBscore 0~2); class 2 (middle, TBscore 3~4); class 3 (severe, TBscore 5~9).

Improvement was defined as a change to a lower degree, i.e. class 3 to class 2 or 1, class 2 to class 1. Those without change or change to a higher category were characterized as no improvement.

Sputum smear and culture and drug-susceptibility testing

Duplicate sputum specimens were collected from the subjects for an acid-fast bacteria (AFB) smear at baseline, weeks 2, 4, 6 and 8. A smear microscopy was performed at the lab in the hospital by using Ziehl-Neelsen staining. All sputum samples at admission had AFB cultures performed with the use of Löwenstein-Jensen solid media and standard methodologies.

Drug-susceptibility testing was performed with the use of the absolute concentration method on solid media with a standard procedure. Multi-drug-resistant tuberculosis (MDR-TB) was defined as resistance to at least isoniazid and rifampicin.

Anti-tuberculosis drug therapy

Standard anti-tuberculosis drug therapy (isoniazid, rifampicin, pyrazinamide, and ethambutol) was started in all subjects based on the diagnosis of tuberculosis. Subjects with confirmed MDR-TB were changed to appropriate second-line drug therapy according to standard clinical care protocol. All subjects received directly observed therapy of anti-tuberculosis drugs and the intervention group received additional vitamin D3 supplements.

Baseline blood chemistry and serial calcium and 25(OH)D concentrations

Blood samples were drawn from each subject after fasting for at least 12 h and an overnight rest. Glucose and lipid indexes were determined immediately after blood was drawn. Glucose oxidase method was used for the determination of FPG using a Hitachi automatic analyzer. Lipid indexes including total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLC) were determined by autoanalyzer under standard procedure. The remaining plasma/serum were stored at -80 ° C until measurement for 25(OH)D. Other biochemical indexes such as aspartate amino transferase, creatinine, calcium, and routine blood test such as white blood cell count, lymphocyte count, etc were carried out by standard method. Plasma calcium concentrations were serially monitored in all subjects at weeks 2, 4, and 8. Patients with co-current diabetes were monitored frequently for blood glucose level in fingertip blood fasting and

two hours after meal. Serum 25-hydroxyvitamin D concentrations were measured with the use of ^{125}I radioimmunoassay (RIA).

Safety monitoring

Criteria for subject discontinuation were established before the intervention. Subjects were withdrawn from the study drug if a plasma calcium concentration was >2.6 mmol/L with signs and symptoms of hypercalcemia during the following 8 wks or if a plasma calcium concentration was >2.9 mmol/L at any time regardless of the presence of symptoms. The subjects were also queried for clinical symptoms that could potentially be related to hypercalcemia with a specific case report form (CRF) once a week continuously for a period of ten weeks. The reported symptoms included stomachache, diarrhea, headache, dizzy, nausea, vomit, joint pain and mental condition.

Statistical Analysis

All the analyses were performed using SPSS version 23.0 software (IBM SPSS Statistics 23). We expressed variables by their means and standard deviations. The Pearson chi-square (χ^2) was used to assess statistical differences in proportions between groups, and the Student t test to assess differences in means between two groups when a normal distribution was present. Log-normal distribution was present in serum 25(OH)D, and the data were log transferred for the t test and 10^x transfer for presentation. Covariance analysis (ANCOVA) was used for the comparison of TBscore between the two groups with adjustment of the baseline TBscore. TBscore was further transferred into three categories namely mild, middle, severe, and improvement rates were calculated as stated in the method section. Multivariate logistic regression (MLR) was used to evaluate vitamin D supplementation on the presentation of improvement. Possible confounders i.e., age and gender was adjusted. Backward stepwise logistic

regression, with entry and removal criteria of P<0.05 and P>0.10 respectively, was used to establish the final multivariate predictive model. Odds ratios and nominal 95% confidence intervals (CIs) are presented. Two-sided P<0.05 was considered significant for all analyses.

Results

A total of 537 patients were assessed for eligibility at the beginning. Excluded were 180 due to: refused to take part in the trial (n=42), HIV infected (n=3), retreatment (n=66), other reasons (n=7). Then 200 patients had additional vitamin D, and 219 were in the control group. During the trial, fourteen patients in the vitamin D group stayed in hospital <2 months, and 12 were drug resistant; while 17 in the control arm stayed in hospital <2 months and 18 were drug resistant. Finally, we analyzed 174 patients in the vitamin D arm (64 patients with concurrent tuberculosis and diabetes, 110 tuberculosis) and 184 in the control arm (73 patients with concurrent tuberculosis and diabetes, 111 tuberculosis). The flow of participants is outlined in Figure 7-1.
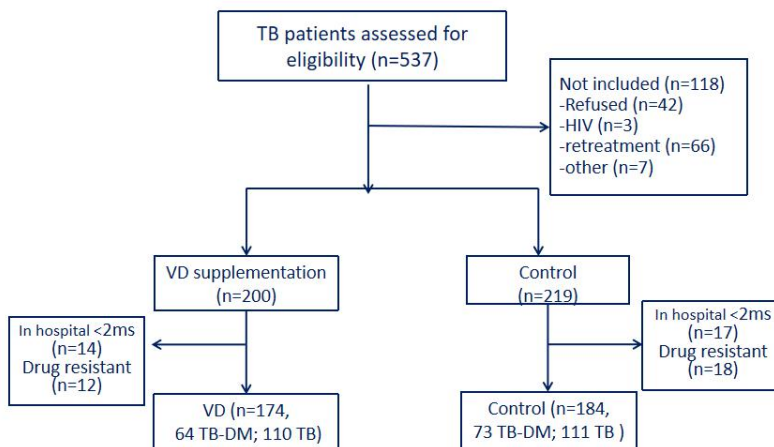

Figure 7-1. Flow of participants in vitamin D supplementation trial

Demographic and clinical characteristics

Demographic and clinical characteristics of the participants were comparablebetween study groups. The prevalence of diabetes, cavity in chest radiograph, positive sputum and the severity of TBscore distributed similarly between the two study groups (Table 7-1).

Table 7-1. Demographic and clinical characteristics of the participants at baseline

	Vitamin D (n=174)	**Control**(n=184)	**P-value**
Age, years, mean(SD)	42.07±15.44	44.12±16.62	0.229
Male, n(%)	137(78.7)	139(75.5)	0.473
SBP	122.20±17.12	121.68±15.67	0.766
DBP	75.32±10.87	76.97±9.32	0.122
BMI	21.41±3.14	21.47±3.19	0.844
<18.5	30(17.2)	34(18.5)	0.571
18.5~23.9	112(64.4)	109(59.2)	
≥24.0	32(18.4)	41(22.3)	
Marriage status			
Married	125(71.8)	137(74.5)	0.576
Unmarried	49(28.2)	47(25.5)	0.234
Smoking history	97(55.7)	91(49.5)	
diabetes	64(36.8)	73(39.7)	0.574
Sputum positive	121(71.2)	120(68.2)	0.545
CT-cavity	80(46.0)	81(44.0)	0.710
TB score	3.93±1.59	3.69±1.55	0.159
Class 1	35(20.1)	41(22.3)	0.801
Class 2	81(46.6)	87(47.3)	
Class 3	58(33.3)	56(30.4)	
Mean 25(OH)D3[†]	6.45±0.21	7.20±0.20	0.394

[†] log×10 transformed for t test between the two groups, and $10^x/10$ for presentation.

Vitamin D status in the participants

The majority of the subjects (95%) was vitamin D deficient. A similar number of subjects in the control (63.5%) and vitamin D (68.6%) groups were severely vitamin D deficient (<10 ng/mL) at baseline. However, vitamin D supplementation did not result in significant increase in serum 25(OH)D in the supplementation group compared to the control (p>0.05).

Adverse Events

During the continuous monitoring of adverse events in the following 10 wks after the start of the trial, the symptoms of adverse effects were reported most frequently at inclusion, at which time 33% reported any of the symptoms before receiving the anti-tuberculosis treatment, the number declined to 25%, 14%, 6% and 5% at 2th, 4th, 6th, 8th wks. Most frequently reported incident adverse effect was nausea (n=31), other side effects included stomachache (n=16), dizzy (n=16), diarrhea (n=13), headache (n=12), mental condition (n=11), vomit (n=9) and joint pain(n=7). No differences of the adverse effects were observed between the vitamin D group and controls, except for diarrhea, with a higher incidence in control group (6.0% vs 1.1%, p=0.015). Also, no cases of hypercalcemia were seen.

Effect of vitamin D supplementation

The TBscore of the intervention and control groups as well as the proportion in moderate and heavy categories (class 2 and 3) at various time points are presented in Figure 7-2. An obvious tendency of ameliorated tuberculosis severity during follow up was observed in both groups, however not significantly different. As shown in Figure 7-3, the percentage improvement (change to a lower severity class) in the vitamin D supplementation group

was borderline significantly higher at weeks 4, 6 and 8 (p=0.083, 0.084 and 0.086, respectively).

Subgroup Analysis by presence of diabetes

Because the co-morbidity of diabetes may have a significant impact on the clinical treatment, we examined the impact of vitamin D supplementation among tuberculosis patients with diabetes and tuberculosis patients without diabetes, separately (n=137, n=221, respectively). Patients with diabetes were older than those without diabetes (52.2±11.1 vs 37.5±16.1, p<0.001), with higher BMI level and higher prevalence of overweight and obesity (29.2% vs 14.9%). Also, concurrent diabetes resulted in significantly higher prevalence of tuberculosis signs and symptoms including positive sputum (78.1% vs 64.7%), cavity in CT scan (55.5% vs 38.5%) and class 2+3 of TBscore (88.3% vs 78.2%).

As demonstrated in Figure 7-4 and Figure 7-6, similar results were observed for both subgroups as in all the subjects shown in Figure 2. We did not find a significant difference between the two arms in both subgroups. Notably, a different profile was observed in the improvement rate of TBscore class in tuberculosis patients with and without diabetes (Figure 7-5 and Figure 7-7). Obviously higher improvement rates were found in the vitamin D supplementation group at 4th, 6th and 8th wks in tuberculosis patients with diabetes (Figure 7-7), while no such effect existed in those without diabetes (Figure 7-5).
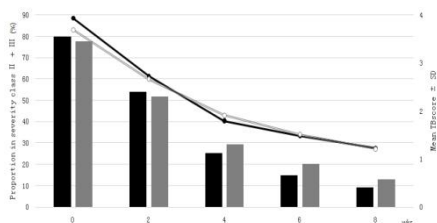
Figure 7-2. TBscore and the severity class various times in all TB patients
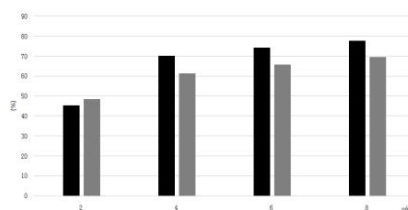

Figure 7-3. Improvement rate of severity class at various times in all TB patients
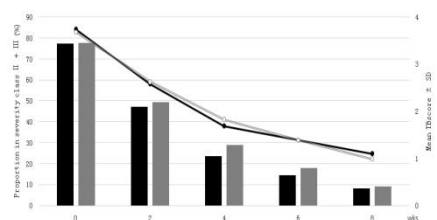

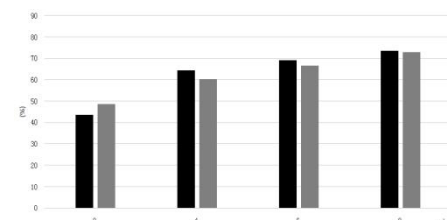Figure 7-4. TBscore and the severity class at various times in TB-only patients

Figure 7-5. Improvement rate of severity class at various times in TB-only patients
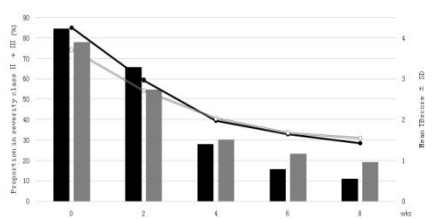

Figure 7-6. TBscore and the severity class at various times in TB-DM patients
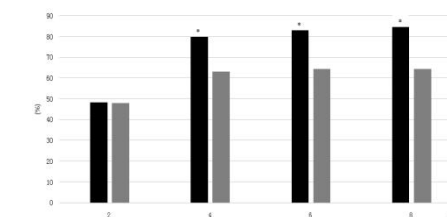

Figure 7-7. Improvement rate of severity class at various times in TB-DM patients

▬▬ vitamin D supplemented   ▬▬ control   ═○═ vitamin D supplemented   ●── control

All TB patients: patients with and without tuberculosis; TB-only patients: tuberculosis patients without diabetes; TB-DM patients: tuberculosis patients with diabetes

In figure 7-2, 7- 4 and7- 6, the fold line showed the mean TBscore of the patients at various times with right axis, and the bar chart showed the proportion of severity class II and III with left axis.

Vitamin D supplementation and improvement of tuberculosis severity

Since difference existed in baseline TBscore between the vitamin D intervention arm and the controls (Figure 7-6), we further analyzed the effects of vitamin D by using multivariate logistic regression.

Tables 7-2 show the results of the logistic regression analysis of factors related to the improvement of TBscore at various time intervals. After adjustment for possible confounders including age and gender, vitamin D supplementation still was related to the improvement of TBscore at the 4th, 6th, 8th week in patients with concurrent diabetes (OR 5.48, 95%CI 1.14-26.40). The odds ratios in the multivariable model were almost the same as the crude one, indicating the effects of vitamin D on the improvement of TBscore were not influenced by age and gender. No similar results were observed in patient with tuberculosis only.

Table 7-2. Multivariable logistic regression of the effect of vitamin D supplementation on the improvement of TBscore at various time intervals

	4th week		6th week	
	Crude OR	Adjusted OR[§]	Crude OR	Adjusted OR[§]
All	1.47(0.95~2.29)[^]	1.50(0.96~2.32)[^]	1.49(0.95~2.36)^	1.51(0.95~2.38)[^]
TB-only	1.19(0.69~2.06)	1.20(0.69~2.07)	1.12(0.64~1.97)	1.10(0.62~1.95)
TB-DM	2.30(1.06~4.98)[*]	2.29(1.05~5.02)[*]	2.66(1.19~5.97)[*]	2.66(1.18~5.99)[*]

	8th week	
	Crude OR	Adjusted OR[§]
All	1.51(0.94~2.44)[^]	1.51(0.94~2.43)[^]
TB-only	1.03(0.57~1.88)	0.99(0.54~1.82)
TB-DM	2.98(1.31~6.83)[*]	2.98(1.30~6.84)[*]

TB-only patients, patients with tuberculosis only; TB-DM patients, patients with concurrent tuberculosis and diabetes; ^$p<0.10$ [*] $p<0.05$; §adjusted by age and gender.

The results of positive sputum smear were presented in Figure 8. Significant decreases were observed with the therapy. In the subgroup analysis, we found that the time for sputum smear conversion in patients with diabetes was obviously longer than those without diabetes. However, no significant difference between vitamin D supplementation and controls was found when analyzed in all patients or by the presence of diabetes (Figure 7-8).
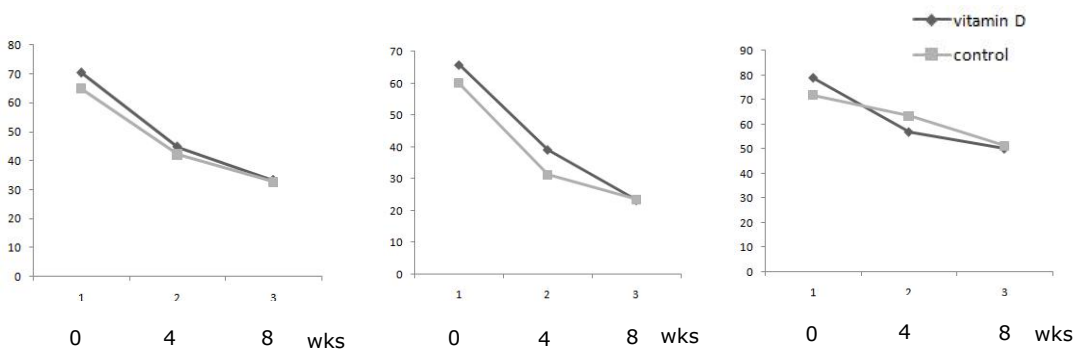

Figure 7-8. The rate of positive Sputum smear at various time

Left: all the subjects; middle: TB patients without diabetes; right: TB patients with diabetes

Discussion

Vitamin D supplementation in tuberculosis patients during intensive-phase treatment did not result in adverse events. The supplementation of vitamin D showed adjunctive effects on the improvement of tuberculosis severity as evaluated by TBscore in patients with combined diabetes and tuberculosis at the 4th, 6th , and 8th week, while no similar effects were found in those

without diabetes. Neither did we find an effect of vitamin D supplementation on sputum smear conversion.

We used an open intervention study design without placebo control in two separate departments: one was the intervention department and one of the other departments acted as an external control group. So, a limitation is that the study was not blind and there was no individual randomization. This design was adopted after discussions with the co-investigators in the hospital. This way contamination of treatment between both groups could be avoided. It was also anticipated that many patients and particularly the more severe cases would be unwilling to run the chance of getting placebo, because of the stigmatization and discrimination of tuberculosis patients that still exist. And the combined diabetes might be associated with worse situation, since more severe signs and symptom at presentation may result in higher sensitiveness in the patients. In addition, the ratio of clinician to patients was approximately 1:7, and the doctors had the responsibility of outpatient service one day a week, which made it difficult to manage the patients in case of individual randomization. However, the diagnosed patients were assigned to each department in turn according to their visiting order, and a large proportion of the patients would stay in hospital for two months receiving the intensive-phase chemotherapy. Therefore, from the perspective of the separate department, patients had a similar degree of clinical signs and symptoms, sharing similar treatment conditions. The intervention was given to eligible patients in one department, while patients from a department that was given standard treatment were observed as a comparison group. Although the study was carried out in two separate departments, clinical therapy, physical examination and face-to-face questionnaire were carried out by using the same procedure, except for the additional vitamin D intervention.

To our knowledge, this is the first vitamin D intervention trial in patients with concurrent tuberculosis and diabetes with sufficient sample size. We did not find hypercalcemia in the subjects during the process of this trial, and the potential signs and symptoms related to hypercalcemia were also not observed. This indicated no significant adverse events due to the supplementation of vitamin D of 800 IU per day during the intensive-phase treatment. We used TBscore for the evaluation of tuberculosis clinical signs and symptoms. The score includes cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, BMI<18 and BMI<16. Change in TBscore has been shown to detect clinical change well; a high TBscore correlates well with mortality and low TBscores correlate with favourable outcomes, cure, and completed treatment [25].

Since in the subgroup of patients with concurrent tuberculosis and diabetes, the vitamin D group had a higher average TBscore than the control group, we considered regression to the mean when setting up the data analysis strategy. We adjusted for baseline TBscore using the continuous variable. However, the odds ratio turned to become inflated instead of diminished by the adjustment. Therefore, we decided to report the odds ratios unadjusted for baseline TBscore. Further analysis will be necessary to elucidate the possibility of regression-to-mean in this study. We further adjusted for age and gender in the model, as the possible confounders. Vitamin D deficiency is more common among older persons [26], and in univariate analysis, a significant correlation with the improvement of TBscore was observed for gender. The adjusted odds ratios were very similar to the crude ones. Therefore, we think vitamin D supplementation had an adjunctive effect from the 4th week onwards during intensive-phase treatment, independent of age or gender.

The possible underlying mechanism of vitamin D supplementation may represent synergistic effects of immunomodulatory[27] and glycemic control [28] of vitamin D. In line with the immunomodulatory effect is our observation that serum 25(OH)D was positively related to leptin, while negatively with TNF-α (chapter 6), indicating the regulation of these immune activity related cytokines[29]. Although the exact pathway of vitamin D on the improvement of the clinical signs and symptoms in patients with concurrent tuberculosis and diabetes needs more research, our results provide an important clue of the role of vitamin D in the adjunctive treatment in patients with combined tuberculosis and diabetes.

No similar effects of vitamin D were found in tuberculosis patients without diabetes, which is in agreement with a recent meta-analysis reporting that the non-skeletal health effects of vitamin D has not been confirmed with sound evidence in randomized trials till now [30]. However, Coussens et al. reported that adjunctive high-dose vitamin D in tuberculosis treatment could accelerate sputum smear conversion, and reduce the inflammatory responses associated with higher risk of mortality[20]. The discrepancy may be due to various factors such as different doses used, different treatment settings. Another explanation may be that the effect in patients with tuberculosis only may be smaller. The number of subjects in this subgroup and the resulting power may have been insufficient to pick up the difference between the supplementation and comparison group. Therefore, the question whether vitamin D supplementation benefits tuberculosis only patients needs further research in an enlarged sample size. A recent study found that the improvement of sputum clearance by high dose vitamin D was modified by SNPs in genes encoding the vitamin D receptor (VDR) and 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) [21]. Therefore, whether vitamin D supplementation has benefits in tuberculosis needs further research to confirm.

Diabetes resulted in more severe presentation, high risk of treatment failure[31] and death[5; 8], and may be associated with increased risk of the development of multidrug resistant tuberculosis (MDR-TB)[32][33]. Chronic hyperglycemia is associated with impaired immunity to *Mtb* in diabetes patients and may result in reduced effectiveness of anti- tuberculosis treatment. In recent years, the double burden of tuberculosis and diabetes in low- and middle-income countries has caused intensive concern of international agencies such as the WHO, International Union against Tuberculosis and Lung disease (the Union) and International Diabetes Federation, to provide strategies upon facing this new challenge. The WHO recommended optimized glucose control as part of the management of tuberculosis and diabetes combined patients for improved tuberculosis outcomes[34]. Till now, widely accepted guidelines for the treatment of tuberculosis patients with concurrent diabetes are not available. Recently, a group of expert clinicians suggested that for patients with combined diabetes and tuberculosis, a total of 9 months of therapy is recommended[35]. Although caution should be taken in the interpretation of the results due to the limitations we have discussed above, our results may open a new therapeutic perspective. We think vitamin D status of the patients should be monitored due to the high prevalence of (severe) vitamin D deficiency in the patients as we previously reported[13]. Outdoor activity to ensure enough sun exposure, higher consumption of fish (especially sea fish), eggs, etc and vitamin D fortified items such as milk, butter is recommended. Vitamin D supplements may have a favourable adjunctive effect on clinical treatment and more confirmative studies are needed in the future concerning the recommendation of vitamin D supplementation as a routine practice. Also, it may benefit tuberculosis patients in general.

In conclusion, for the first time, we identified a favourable adjunctive effect of vitamin D on the clinical manifestations in tuberculosis patients with diabetes during the intensive-phase treatment. Our results may open a new perspective of the treatment of the combined diseases. However, partly due to methodological limitations of our study, we could not claim effects of vitamin D with certainty at this stage. The results of our study need to be confirmed in the future.

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8 General discussion

The research described in this thesis aimed to investigate the prevalence of diabetes in active tuberculosis patients, to compare the risk of having diabetes between persons with and without tuberculosis, and to elucidate the role of vitamin D deficiency in the combined occurrence of these diseases from an epidemiological perspective.

Main findings

The prevalence of diabetes in patients with active tuberculosis in rural areas of China was 6.3% which was nearly 35% higher than in non-tuberculosis controls. Adjusted for confounders, tuberculosis patients had a slightly more than three times higher risk of having diabetes (OR 3.17, 95% CI 1.14-8.84). Risk factors for diabetes and pre-diabetes among tuberculosis patients were similar as observed in the general population in China, i.e., older age, overweight and obesity, higher income level (chapter 2).

Diabetes and pre-diabetes were also associated with a nearly two-fold higher probability of patient delay of tuberculosis in the community (chapter 3). In addition, we found that compared with tuberculosis only patients, those with combined diabetes had a higher prevalence of positive sputum (78.1% vs 64.7%), of pulmonary cavity in CT scan (55.5% vs 38.5%) and of a more severe class of clinical signs and symptoms including cough, hemoptysis, dyspnea, chest pain, night sweating, etc. as indicated by severe

TBscore class (88.3% vs 78.2%) at admission (chapter 7). Notably, among diabetics an increased risk of delayed sputum conversion was observed, indicating higher risk of transmission of tuberculosis in the community.

A high prevalence of vitamin D deficiency [serum 25(OH)D<20ng/mL] of nearly 80% was observed in tuberculosis patients in our study. Risk factors identified for deficiency were severe tuberculosis symptoms at presentation, low outdoor activity and low fish consumption (chapter 4). Compared with patients in the highest quartile (≥26.62ng/mL), those in the lowest quartile of serum 25(OH)D (≤8.57ng/mL) had a 4.73 and 2.50 fold increased risk of having tuberculosis, and tuberculosis combined with diabetes, respectively, after adjustment for potential confounders (chapter 5).

Vitamin D status was inversely associated with TNF-α, while positively associated with leptin, cytokines that are closely related to anti- *Mtb* immune activity in the body, indicating a possible mechanism of vitamin D in the pathogenesis of tuberculosis (chapter 6). The supplementation of vitamin D showed an adjunctive effect in the clinical improvement at the 8th week during the intensive-phase treatment in patients with the combined diseases, after adjustment for confounders, i.e., age and gender (chapter 7).

Underweight was related to hyperglycemia in tuberculosis patients, having a complex correlation with the risk of having the combined disease in this study. Furthermore, a synergistic interaction on the risk of having combined tuberculosis and diabetes was observed between underweight and low serum 25(OH)D (chapter 2 and chapter 5).

Internal validity

Study design

We carried out observational studies (chapter 2-6) and an intervention study (chapter 7) in this thesis. The interpretation of the results of the cross-sectional studies should be done with caution. Concerning variables that are not subject to change such as age, gender, race, causal relationships can more readily be inferred. However, as to other variables such as vitamin D status, we cannot ascertain the sequence of occurrence and reverse causality may also explain the results. In addition, despite adjustment for possible confounders in data analysis, residual confounding cannot be completely ruled out. In addition, as discussed in chapter 7, a design of an open intervention with an external control group from the same hospital was adopted in the vitamin D supplementation trial. These limitations need to be considered when making causal inference based on the results.

Study Population

Recruitment

The study population in chapters 2, 3 and 5 was recruited from a rural area in Linyi, located in Shandong province in North China. The patients were consecutively included from seven tuberculosis clinics, that each had a defined catchment area comprising approximately 0.9 million inhabitants. We recruited subjects from county-level tuberculosis clinics where patients from rural communities are usually diagnosed and treated following the DOTS principle. Therefore, representativeness for the general population can be assumed. Cluster random sampling was used to recruit non-tuberculosis controls from the same communities as the tuberculosis

cases, stratified for economic status, since people living in economically developed regions have a higher prevalence of diabetes in China[1]. This way, comparability of tuberculosis patients and non-tuberculosis controls could be assured.

The subjects in the patient delay study were a subpopulation from the previous study. We randomly selected 2280 tuberculosis patients from this study to analyse diagnosis delay and its risk factors (chapter 3). Also, the subjects in chapter 5 were a subset from the previous large-scale diabetes screening study. Subjects were randomly selected from the four groups including tuberculosis-only patients, tuberculosis patients with diabetes, diabetes-only patients and normal controls. The left-over serum after determining biochemical parameters (i.e. glucose level, lipid profile) was used to detect 25(OH)D. Due to an insufficient amount of serum left, finally, 130 tuberculosis, 90 tuberculosis-diabetes, 91 diabetes and 134 normal controls were included in the analysis for serum vitamin D concentrations (chapter 5). The demographic and clinical characteristics at baseline of the included subjects were compared with those excluded, and no significant difference existed between them. Therefore we are confident that the exclusion did not give rise to selection bias.

In chapters 4, 6, and 7, we recruited tuberculosis patients from a city level specialised tuberculosis hospital located in Qingdao, Shandong province of China. This hospital has a catchment area of about 9 million people, and the average number of hospitalized patients with tuberculosis was about two thousand cases per year. In chapter 4, 461 active tuberculosis patients aged 16~86 years were randomly selected including 192 tuberculosis with diabetes and 269 tuberculosis without diabetes. In chapter 6, a total of 299active tuberculosis patients were randomly recruited also from this tuberculosis hospital, together with 91 normal controls recruited from a physical check-up population in a general hospital in the same city. For

chapter 4 and 6, there is an overlap of 70% between the tuberculosis patients, which were recruited from the same tuberculosis hospital. In chapter 7, 358 newly diagnosed pulmonary tuberculosis patients aged ≥18 y, staying in the hospital of 8 or more weeks were included from the 461 patients in chapter 4, with 174 cases in VD3 supplementation group and 184 in control group.

Statistical efficiency

Sample size calculation was carried out in the design stage of our study. For the diabetes prevalence investigation (chapter 2), a sample size of 7000 tuberculosis patients was calculated assuming a diabetes prevalence of 6.7% among tuberculosis subjects as reported in the literature [2]. We considered a non-response rate of 20%, and assumed an estimated power of 80%. We calculated the required number of tuberculosis patients and controls using the formula,

$$N = \frac{z^2 \times PQ}{d^2}$$

P is the prevalence of diabetes in tuberculosis patients (6.7%), Q=1-P, d is tolerance error, d=0.1×P and z was taken as 1.96 conform a two-sided significance level α=0.05.

We later adjusted it to 6200 subjects based on the favourable compliance in a pilot study in two communities in this area. Finally, 6382 active tuberculosis patients and 6674 non-tuberculosis controls were included in the analysis, which meets the sample size requirement. Similarly, we estimated the number of tuberculosis patients needed in the vitamin D deficiency prevalence study by using a power calculation based on a study

from Korea [3], a country at similar latitude, reporting 51.5% vitamin D deficiency in tuberculosis patients. An estimated drop-out rate of 10% was considered. The number of tuberculosis patients needed to be investigated was 420 with an estimated power of 80% and the tolerable error of 10% (chapter 4). Finally, 461 active tuberculosis patients aged 16-86 years were recruited.

In conclusion, we consider the included study populations as representative for their source populations, and we are confident that the sample sizes were sufficient for the purpose of the studies.

Field work model for the multi-centre investigation

As we described in chapter 2, a large-scale epidemiologic study was carried out to screen diabetes in active tuberculosis patients. This is a multi-centre investigation in various county-level tuberculosis clinics in Linyi. Therefore, it was of great importance to set up an efficient field work scheme. The model of field work is shown as Figure 8-1.

Firstly, the core members of the team examined the possible candidate clinics with respect to the efficiency of the anti-tuberculosis system, the number of potentially reachable active tuberculosis patients, the qualification of the clinic lab, etc. If the conditions were fulfilled, an agreement was signed. Subsequently, investigation started including training of co-investigators, screening diabetes in tuberculosis patients, and administering of the dietary and lifestyle questionnaire, as well as health promotion in the patients and the lay public. During the implementation of the field work, the core team members from Qingdao University were on site nearly 10 days a month, working together with the co-investigators. Workshops and seminars were held frequently during the project. Any

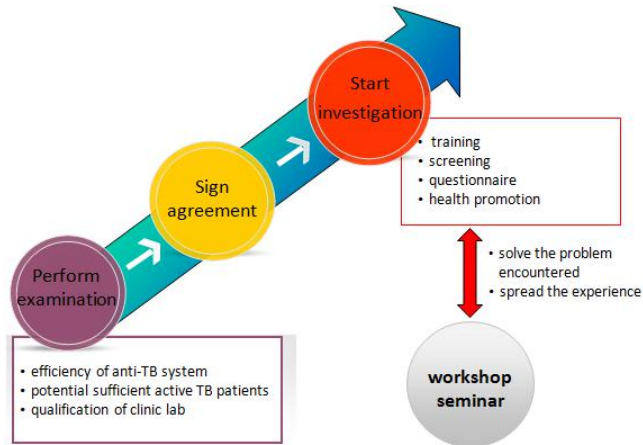problem encountered was discussed promptly, and experience gained was spread to other centres in time.

Figure 8-1. The field work model for the multi-centre investigation.

A standardised process was practiced in this multi-centre investigation. The organization of the screening was based on the "county—township—village" tuberculosis control system. The county level tuberculosis clinics played a core role in the screening. A collaborative working team was established during this project.

Training

The training was conducted in a stepwise manner in this project (top level-second level-third level, see in Table 8-1). The trainees included not only co-investigators (the clinicians from seven county tuberculosis clinics who would directly take part in the project), but also physicians in local general hospitals specialized in the treatment of diabetes, and village nurses working in the first-line health service unit. We expanded the scope of the trainees mainly due to the poor awareness level of the double burden in this

rural area which was recognized in the pilot study. In total, nearly 70 tuberculosis workers and 260 clinicians of internal medicine and other related domains took part in the top-level training; 120 tuberculosis workers in the counties and 200 in the township health centre attended the second-level training and about 2000 village nurses were trained (third-level). The training strategy was designed according to the "county-township-village" three-level anti-tuberculosis network, which can cover almost all the local tuberculosis workers. It turned out to be an effective training method in the rural area, although the training of the village nurses still faces challenge.

Table 8-1. Training scheme of the project

Level	Participants	Training model	Faculty
Top-level	Tuberculosis workers in charge of the field work of each community; internist, etc.	Concentrated training	Three from the project team, one from the bureau of disease control, two pulmonary tuberculosis experts, two diabetes experts
Second-level	Tuberculosis workers from the county tuberculosis dispensary; county chest hospital; township health center	Concentrated training	One from the project team, two trainees from the top-level training course
Third-level	Village nurses	Concentrated training, panel discussion, etc.	Three trainees from the second-level training course

The diagnosis of Tuberculosis

The diagnosis of tuberculosis was made within the existing tuberculosis prevention and control system in China, following the criteria provided by WHO. Clinical manifestations, sputum smear microscopy and chest radiography are the central components of the diagnosis [4]. Suspected tuberculosis cases were investigated by sputum smear examination. If positive, the patient was diagnosed with smear-positive tuberculosis; if negative, but the chest radiograph was compatible with active tuberculosis, the patient was diagnosed with smear negative tuberculosis after discussion between clinical and radiographic specialists. In the local clinical setting of our study, *M. tuberculosis* infection was generally not confirmed by sputum culture. Anyhow, the national tuberculosis epidemiological survey in China reported that culture confirmation is present in only 26.4% of the active tuberculosis patients.

Patient delay

A health seeking interval between the onset of tuberculosis symptoms and the first visit to a county-level tuberculosis dispensary > 28 days was defined as patient delay in our study (chapter 3). The onset of symptoms relied on self-report, which may have led to error. However, the diagnostic procedures in this area were uniform and standard methods were used. The socio-demographic variables, including income, education level, and type of health facility consulted by tuberculosis patients, were similar across participants with and without diabetes, which will have balanced the possible information error to a large extent and will not have biased the difference between diabetics and non-diabetics.

The diagnosis of diabetes

Diagnosis of diabetes and pre-diabetes was according to WHO criteria for the classification of glucose tolerance based on fasting plasma glucose (FPG) [5]. After an overnight fast, venous blood of each participant was collected. Glucose oxidase method was used to estimate FPG level. Those with FPG≥6.1 mmol/L were referred to diabetes clinics for diagnostic confirmation with a second FPG test. Those with FPG level in the range of 6.1 mmol/L to 6.9 mmol/L were diagnosed as pre-diabetes; those with FPG level ≥7.0 mmol/L were diagnosed as diabetes. We collected blood from newly-diagnosed tuberculosis patients and non-tuberculosis controls from the same community after fasting overnight to determine plasma glucose concentration. In a considerable number of studies into the double burden of tuberculosis and diabetes reported in the literature [6; 7; 8], the prevalence of diabetes was calculated using secondary data from the diabetes surveillance system or relying on self-report, in which inherent biases cannot be avoided. Also, in these studies, diabetes prevalence was not investigated in non-tuberculosis controls from the same source population, which may have led to impaired comparability.

We chose a suitable moment to detect diabetes in the patients. Tuberculosis, as a chronic infectious disease, was recognized to induce transient hyperglycaemia [9]. Therefore, over-diagnosis of diabetes might result if testing would take place prior to initiation of tuberculosis treatment. Since most of our study subjects were screened for diabetes about 1–2weeks after the initiation of tuberculosis therapy, this will not have biased our results to a large extent. Inter-lab comparison of glucose measurement was carried out frequently to ensure the reliability of the data.

However, diabetes screening as carried out in our study also has its limitations. Oral glucose tolerance test (OGTT) was not performed in the participants. The main reason was that most of the participants lived in rural

villages, usually far away from the tuberculosis clinics located in the county. This made OGTTs impractical. The prevalence of diabetes in the study population may consequently have been underestimated. Among Asian patients with diabetes, isolated hyperglycaemia two hours after glucose loading is common [10]. A national study in China found 46.6% of the cases of undiagnosed diabetes met the criteria for elevated 2-hour plasma glucose levels in an oral glucose-tolerance test but not the criteria for elevated fasting glucose levels [11]. We screened diabetes in tuberculosis patients and controls by using identical procedure, which would lead to the same level of underestimation and therefore leave the relative odds more or less unaffected. However, the relatively low sensitivity of the procedures may have resulted in an unknown bias in the odds ratio estimation.

The measurement of serum 25(OH)D concentrations

Serum 25(OH)D concentrations in this study were measured in Beifang Institute of Biotechnology (Beijing, China) by using the method of ^{125}I radioimmunoassay (RIA). The kit from DiaSorin Inc (Stillwater, Minnesota, USA) was used. For quality control, two reference controls provided with the kit were utilized (one at low-normal level and one at high-normal level) to guarantee assay performance. The sensitivity of the assay was 1.5 ng/mL. The inter-assay variability was 10.5% and the intraassay variability was 8.2%.

To characterise the participants with respect to vitamin D deficiency, the most widely accepted criteria [12] were used in our study. Serum 25(OH)D of 10ng/mL and 20ng/mL was used as the cut-off value of severe vitamin D deficiency and vitamin D deficiency respectively. In chapter 5, we used quartiles of the distribution of serum 25(OH)D to divide the 445 subjects into four groups, and analysed the risk of tuberculosis-only, tuberculosis combined with diabetes and diabetes only in the lowest quartile (≤ 8.57

ng/mL) compared with the highest quartile (≥26.62 ng/mL). The main reason for using quartiles here was to ensure sufficient number for each subgroup to estimate the odds ratios.

Control of Data quality

We took various actions to ensure the reliability of the data in this epidemiological study. As previously stated, a standardized procedure of diabetes screening was used in the multi-centre investigation. All co-investigators were trained before the screening, and core team members worked in different sites to supervise the implementation of the project.

The diagnosis of tuberculosis and diabetes was carried out by using the widely accepted criteria, and regular calibration of medical devices for the determination of blood glucose was carried out. Serum vitamin D levels were determined by standard method. Information on potential confounders such as smoking, drinking, physical activity of the subjects was collected by using a face-to-face questionnaire, and the reliability and validity of the questionnaire was tested before being used in the field. Height and weight of the subjects were measured by standard procedure to calculate BMI.

For data analysis, double data entry was carried out, and then a computer-based error detection system was used to check the consistency of the data. In case of inconsistency, the original questionnaire was checked and the error corrected via a re-entry.

Confounding

Major potential confounders such as BMI, age and income level [13; 14] were considered in the present study. To eliminate the effects of confounders, we took account of them in different stages of our study, including design, data collection and data analysis.

-Design   At the inspection of the probable site, we found that the economic status differs in different towns in the community. Therefore, we considered economic status when we selected the non-tuberculosis controls. We divided economic status into high, middle and low according to report of local residents' income level and used stratified cluster random sampling to recruit the non-tuberculosis controls to ensure similar economic status.

-Data collection   Information on the following potential confounders has been collected by structured face to face interviews: economic status, environmental situation, food resource, dietary pattern, marital status and health-related behaviors including cigarette smoking, alcohol drinking, physical activity of the subject. Height and weight of the subject were measured to calculate BMI. Since season of the year is also an important factor that influences vitamin D status in the body, we collected the date of blood sampling in order to be able to adjust for season in later data analysis. Nevertheless, we could not collect the individual's waist-to-hip ratio [15], which may lead to certain bias in analyzing the risk factors of diabetes in tuberculosis.

-Data analysis   Multivariable analyses were performed to adjust for possible confounders in data analysis. The variables for inclusion in the multivariate model were chosen based on plausibility and a p value of <0.1 in univariate analysis. All variables were checked for collinearity in the multivariable models. In the vitamin D supplementation trial, possible confounders including age and gender were adjusted.

In conclusion, we are confident that we have adequately adjusted for potential confounders in our study. We took various methods to guarantee the quality of the data collected including the confounders. Therefore, although residual confounding can never be completely ruled out, we think we minimized it as far as possible in our study.

External validity

The double burden of diabetes and tuberculosis

The screening of diabetes revealed a nearly three-fold higher risk of having diabetes among tuberculosis patients compared with controls. Reports of diabetes prevalence in tuberculosis patients in the literature [16], vary from 1.9% to 45% with high levels reported in South Asia and the Pacific. However, large diversity exists in screening methods, care centres, and study designs across studies. To our knowledge, few studies used primary data with comparison to non-tuberculosis controls in the rural setting, as we did. Therefore, our data fill an important gap in describing the double burden of tuberculosis and diabetes. Our finding is in line with another study in eastern rural area in China that reported a prevalence of 7.7% of diabetes in 1252 tuberculosis patients [17].While other studies in urban areas in China found higher prevalence, from 11.9%~30.8% [18; 19; 20; 21; 22].

The difference in prevalence of diabetes among tuberculosis patients between rural and urban areas is in line with the difference in the general population [23] . This suggests that both populations may share conventional risk factors such as older age, family history of diabetes as we found in our study.

A study in a rural area in India reported 27.5% diabetes prevalence in tuberculosis patients [24]. However, the data in this study was drawn from medical records, coming from a small sample size of 192 tuberculosis patients. Another study conducted in southern India reported that the prevalence of diabetes and pre-diabetes in tuberculosis patients was 25.3% and 24.5%, respectively [25]. In the similar setting of the double burden of tuberculosis and diabetes, like China, India is also witnessing a rapid increase in the incidence of diabetes in recent years, and a high burden of tuberculosis that ranks first in the world. From the border area between

Texas and Mexico, a study reported that the prevalence of diabetes in tuberculosis patients was 39% and 36%, respectively [26].

In conclusion, these results together with our findings indicate a significant double burden of tuberculosis and diabetes which may have important implications for public health in low-and middle-income countries.

We found the peak age of onset of tuberculosis combined with diabetes to be ≥50 years, indicating that older age is a risk factor of the combined disease. This coincides with the incidence of diabetes and indicates that diabetes may be the primary event in the combined occurrence of both diseases as widely claimed in the literature [27].

Nearly 80% tuberculosispatients have a health-seeking delay in our study, which was higher than some previous studies [28]. The higher delay in the present study may be due to different definitions of patient delay and the different study populations as we stated in chapter 3. We suppose that the low education level may have contributed partially to the high patient delay, which was also reported by others [29] [30]. Notably, we found that hyperglycaemia, including diabetes and pre-diabetes, was associated with a nearly two-fold higher probability of patient delay. This suggests that diabetes may also facilitate the transmission of tuberculosis. The few available studies concerning the effect of coexisting diabetes on patient delay observed similar results. Another study in China reported that tuberculosis patients with diabetes had a significantly longer median diagnostic delay than those without diabetes (25 days vs. 6 days), and diabetes was associated with more serious clinical presentations among tuberculosis patients [31]. Studies in intermediate-to-low tuberculosis incidence settings reported similar findings [32] [33]. A recently reported retrospective observational study in 133 patients in a low incidence setting

of Melbourne found that diabetes mellitus was related to prolonged patient delay (OR 3.02, 95% CI 1.04, 8.78), while the median patient delay [28 (0-61) days] was shorter than the present study [32]. However, a study in India investigated the factors associated with health systems delay and diabetes was not found to be a significant factor [OR, 95% CI: 1.26 (0.89–1.78)] [34].

At the same time, diagnosis delay is closely related to a more severe clinical presentation and poorer prognosis in tuberculosis patients. Therefore, the association between diabetes and patient delay is assumed to aggravate both the individual and public health burden.

High prevalence of vitamin D deficiency in tuberculosis patients

We reported a high prevalence of vitamin D deficiency (83.1%) and severe vitamin D deficiency (47.1%) in active tuberculosis patients, and the median of vitamin D was 8.50 ng/mL. Another investigation in Korea, located at a similar latitude as our study area, reported that tuberculosis patients had a low median 25(OH)D concentration of 9.86 ng/mL, which was close to our findings [3]. A study in an equatorial population found that mean serum 25(OH)D in tuberculosis patients was 34.64 ng/mL and the deficiency rate was 41.2% [35]. From an ecological epidemiologic viewpoint, latitude is an important factor of vitamin D status. This is an illustration of the fact that sun exposure is the main source of natural vitamin D in the body.

Tuberculosis symptoms, outdoor activity and fish consumption were found to be associated with the prevalence of severe vitamin D deficiency. The severity of initial tuberculosis signs and symptoms showed a negative association with serum 25(OH)D and patients with a severe tuberculosis score class had a nearly 5-fold higher odds of severe vitamin D deficiency compared with those with a mild class. This provides further evidence for the perspective that vitamin D deficiency is associated with higher risk of

tuberculosis. Decreased appetite or diet limitation in certain populations results in lower food intake including sea fish that may contain some vitamin D.

Being a typical chronic wasting disease, tuberculosis will lead to restricted outdoor activity, which contributes to the situation. Also, severe weight loss, decreased appetite and food intake may result in nutrients depletion in general and vitamin D in particular. Tuberculosis patients obviously have reduced adipose tissue, which may result in diminished vitamin D reserves [36]. Furthermore, vitamin D requirement increases in tuberculosis, since vitamin D plays a key role in anti-tuberculosis immunity. It binds to toll-like receptors on macrophages leading to increased production of 1,25(OH)2D from 25(OH)D [37], which is a key step in the induction of anti-*Mtb* peptide in macrophages. The increased need of vitamin D in tuberculosis patients may also contribute to lowered serum 25(OH)D concentrations.

However, at this stage we cannot tell whether it is vitamin D deficiency that induces tuberculosis or vice versa. Further research is needed to find out the direction of causality in the relationship between vitamin D deficiency and the incidence of active tuberculosis.

BMI in relation to combined diabetes and tuberculosis

The role of BMI in the setting of co-morbid diabetes and tuberculosis is complex. Underweight (BMI<18.5 kg/m^2) showed an increased risk of pre-diabetes and a borderline significant increased risk of diabetes among tuberculosis patients in our study. This finding was in line with a study carried out in Tanzania [13] that reported an association between severe underweight (BMI<16 kg/m^2) and diabetes among male tuberculosis patients (OR 2.52, 95% CI 1.34~4.74, p = 0.004). However, the number of

subjects with both characteristics was relatively small in that study (n=23). Notably, accumulating evidence has indicated the potential adverse influence of underweight on cardiometabolic milieu. An U-shaped relationship was observed between BMI and all-cause mortality [38], and low BMI was associated with adverse outcomes of coronary heart disease in an Asian population [39]. Moreover, a large-scale cohort study in Japan showed that underweight may be associated with the risk of type 2 diabetes among older adults aged 60–79 years [40]. Alternatively, reverse causality may also exist. Poorly controlled glucose in diabetes patients may result in weight loss. On the other hand overweight and obesity are protective against tuberculosis [14]. Weight loss due to poorly controlled diabetes and metabolic de-compensation may take away this protection. Therefore, the interaction between BMI, diabetes and tuberculosis may work out differently in different individuals [41].

Moreover, we found a synergistic effect of underweight and low serum 25(OH)D on the risk of having concurrent diabetes and tuberculosis. Therefore, subjects with both characteristics may have a higher risk. The correlation between BMI and vitamin D status in tuberculosis patients has been inconclusive till now. We found that BMI had a borderline positive association with increased odds of severe vitamin D deficiency (chapter 4), which is in accordance with existing reports that an inverse correlation exists between circulating 25(OH)D concentration and BMI in the healthy population, in overweight/obese subjects and in diabetic patients [42; 43; 44; 45; 46; 47; 48]. It was speculated that lower vitamin D status in obesity is due to a greater pool of distribution [49]. However, there is still insufficient evidence in tuberculosis patients, and the available data diverge. Like our study, Friis et al. reported that tuberculosis patients with lower BMI had higher serum 25(OH)D [50]. However, an investigation in 105 tuberculosis patients found that vitamin D deficiency is more obvious in those with low BMI [51], and BMI

is positively associated with serum 25(OH)D [35]. The main reason for the discrepancy may be differences in sample size and inclusion criteria among those studies. Therefore, the correlation between BMI and vitamin D status in tuberculosis needs further study in larger populations.

Vitamin D deficiency and the double burden of tuberculosis and diabetes

We found a negative association between serum 25(OH)D and prevalent tuberculosis with and without diabetes, which is in accordance with other reports [52]. In addition, the supplementation of vitamin D was observed to have an adjunctive effect on the improvement of clinical manifestations at 8th week in tuberculosis patients with concurrent diabetes, while no similar result was found in those without diabetes.

Nonetheless, contrary to expected, 25(OH)D concentrations, whether analysed as a continuous variable or categorized variable (grouped as quartile levels) were not significantly associated with the prevalence of diabetes (chapter 5). In recent years, abundant epidemiological studies indicate the correlation between vitamin D deficiency and higher risk of type 2 diabetes, including some prospective studies [53; 54]. However, the possibility of reverse causality in observational studies was brought up [55], and the stated correlation has not been verified in randomized controlled trial till now [56].Similar to our study, a 5-year follow-up study found that low 25(OH)D status was not significantly associated with incident diabetes [57]. In addition, due to the cross-sectional design in chapter 5, we cannot infer causality. Therefore, more longitudinal investigations are needed in the future. Furthermore, the odds ratio for prevalent tuberculosis with diabetes comparing the lowest with the highest quartile [OR (95% CI) = 2.50 (1.04-6.02)] was clearly lower than the same odds ratio for prevalent

tuberculosis [OR (95% CI) = 4.73 (2.04-10.9)] (chapter 5). Tuberculosis patients had a significantly lower level of serum 25(OH)D than diabetes patients (12.2 vs 17.86 ng/mL). This may partially explain the result. In addition, in a later study (data not shown), we noticed that the prevalence of severe vitamin D deficiency (serum 25(OH)D <10ng/mL) was higher in patients with combined tuberculosis and diabetes compared with those without (28.4% vs 16.0%). Moreover, a higher prevalence of severe vitamin D deficiency in diabetics than in normal controls (10.0 vs 3.3%) was observed. Integrating these results, we deduce that there may be a threshold effect of vitamin D deficiency in the body. Although we cannot determine the optimal cut-off value at this stage, our data provided an important clue and further study in a larger population is needed, especially concerning combined tuberculosis and diabetes.

Interestingly, patients with concurrent tuberculosis and diabetes had significantly lower serum 25(OH)D which was approximately 60% of that in diabetes (10.58 vs 17.86 ng/mL) (chapter 4). Since diabetes usually comes first, and vitamin D deficiency is prevalent in the general population [58; 59; 60], this result provides evidence for our working hypothesis that vitamin D deficiency may increase the risk of tuberculosis mediated by diabetes. Therefore, low serum 25(OH)D levels in diabetes patients, particularly serum 25(OH)D of lower than 10ng/mL may indicate higher risk of tuberculosis in this population.

We found different results for serum 25(OH)D concentrations (medians) in chapter 4 versus chapter 5 in patients with tuberculosis and diabetes combined (10.58 vs 12.90 ng/mL), as well as in patients with tuberculosis only (7.26 vs 12.20 ng/mL). Although different detection batches may have some impact, the same detection process and kits from the same manufacturer were used in this study. The seasons for blood samples collection were distributed similarly in the two investigations, which may

have minimized the influence of season. As we have discussed previously, the study population in chapter 4 was recruited from a city level tuberculosis hospital, while in chapter 5 from a rural area. We propose two explanations for the difference. Firstly, most of the patients in rural areas were farmers, who had more opportunity of sun exposure, the main source of natural vitamin D in human body. Secondly, the inpatients recruited from city level tuberculosis hospitals usually had more severe tuberculosis and consequently less physical activity than the domestically treated patients.

The supplementation of vitamin D3 in patients with combined diseases showed significant effects on the improvement of TB manifestation at 4th, 6th, 8th week, adjusted for confounders as age, gender. The severity of clinical manifestation in tuberculosis patients was evaluated by TBscore, a measure based on tuberculosis related signs and symptoms, including cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, BMI<18 and BMI<16. Change in TBscore has been shown to detect clinical change well; a high TBscore correlates well with mortality and low TBscores correlate with favourable outcomes, cure, and completed treatment [61]. To our knowledge, this is the first vitamin D intervention trial in patients with combined tuberculosis and diabetes with sufficient sample size. The underlying mechanism may be due to the synergistic effects on the regulation of immune activity [62] and glycaemic metabolism [63] of vitamin D in the body. Also, more severe baseline TB signs and symptoms in patients with diabetes may lead to a more obvious effect of the supplementation. Although the exact pathway of vitamin D related effects needs more research, our results provide an important clue of the role of vitamin D in the adjunctive treatment in patients with combined tuberculosis and diabetes. As reported, diabetes resulted in higher risk of treatment failure [64] and death [65; 66], which urgently calls for an efficient

treatment strategy. Therefore, our results may open a new therapeutic perspective. However, due to the limitations of the intervention trial as we discussed in chapter 7, we should be cautious to the interpretation of the effects of vitamin D.

No similar effects of vitamin D were found in patients without diabetes, which is in agreement with a recent meta-analysis reporting that the non-skeletal health effects of vitamin D have not been confirmed with sound evidence in randomized trials till now [56]. However, Coussens et al. reported that adjunctive high-dose vitamin D in tuberculosis treatment could accelerate sputum smear conversion, and reduce the inflammatory responses associated with higher risk of mortality [67]. The discrepancy may be due to various factors such as different doses used, different treatment settings. A recent study found that the improvement of sputum clearance by high dose vitamin D was modified by SNPs in genes encoding the vitamin D receptor (VDR) and 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) [68]. In addition, the power of the statistical analysis in patients with TB only may have been lower than anticipated, which means the sample size in the trial may have been insufficient to pick up the difference between the supplementation group and the control. Therefore, whether vitamin D supplementation has benefits in tuberculosis needs further researches in an enlarged sample size.

Overall conclusions

The risk of having diabetes in active tuberculosis patients was nearly three times higher compared to non-tuberculosis controls. In the concurrent cases, diabetes frequently precedes tuberculosis, and age ≥ 50 years, male gender in diabetics are associated with increased risk. Severe hypovitaminosis D was prevalent in tuberculosis patients, the main determinants being severe

TBscore class in presentation, low outdoor activity and inadequate fish consumption. Patients with combined diabetes and tuberculosis had a higher percentage of severe vitamin D deficiency, and in this subpopulation vitamin D supplementation in the intensive-phase treatment (8 weeks after anti-tuberculosis therapy) showed an adjunctive effect in the improvement of tuberculosis clinical manifestations at the 8th week. Regulation of immune activity related cytokines such as leptin and TNF-α may be one of the possible mechanisms of vitamin D in the body, together with the synergistic effects of vitamin D on glycaemic metabolism. Our results indicate an aggravating role of vitamin D deficiency in the double burden of tuberculosis and diabetes, and the possible adjunctive effects of vitamin D supplementation.

Implications and recommendations

The double burden of tuberculosis and diabetes in low- and middle-income countries such as India, China, Indonesia in recent years have caused intensive concern of international agencies such as the WHO, the Union (International Union against Tuberculosis and Lung disease) and International Diabetes Federation, and urge them to provide strategies for facing this new challenge. Diabetes has been recognised as a moderate to strong risk factor for active tuberculosis[69] [70] [71] [72]. This has important public health implications. Besides tripling the risk of active tuberculosis [6], diabetes was suggested to have an all-around effect on the natural course of the disease, including a higher risk of the individual becoming infected (latent infection) [73], a higher lifetime risk of tuberculosis activation, and an unfavourable clinical course including presentation of more symptoms, more relapses, treatment failures and deaths [65] [74]. In addition, vitamin D

deficiency may strengthen this association [75]. Our study provided further valuable evidence in a rural setting in China suffering from a typical double burden. In a community based large-scale investigation, we found a higher risk of having diabetes and vitamin D deficiency in active tuberculosis patients, and the combination of tuberculosis with diabetes resulted in higher proportion of patient delay. Also, we carried out a study in a city level tuberculosis hospital. In agreement with other reports, more severe tuberculosis manifestation at presentation was observed in patients with the combined diseases. The supplementation of vitamin D showed a possible adjunctive effect on the improvement of clinical signs and symptoms in this comorbid subgroup. As shown in Figure 8-2, we infer that diabetes may increase the risk of tuberculosis, resulting in higher possibility of tuberculosis transmission in the community. In addition, we speculate vitamin D deficiency may play an aggravating role in this process.
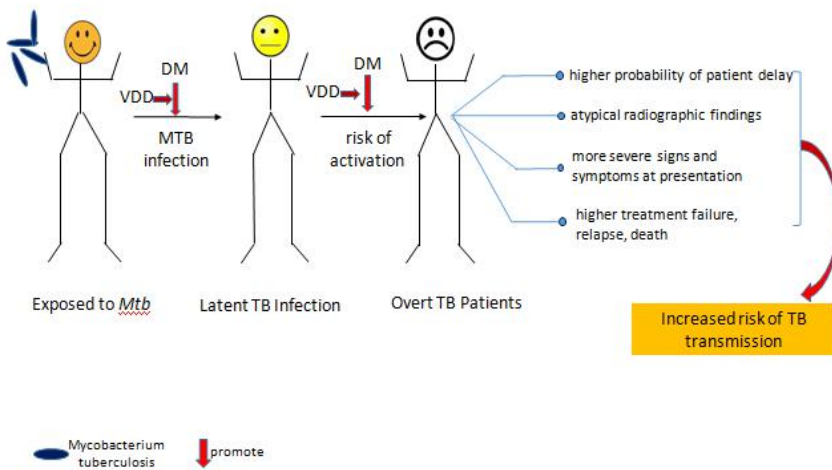

Figure 8-2. The impact of diabetes on tuberculosis epidemic and the role of vitamin D deficiency

Therefore, it is urgent to provide strategies to cope with the emerging challenge of the double burden of tuberculosis and diabetes. The following actions may have significant public health benefits.

Bidirectional screening for tuberculosis and diabetes

"Bidirectional screening" in the thesis means screening for tuberculosis in diabetes and the reverse. It was demonstrated that tuberculosis prevalence in diabetes is high, ranging from 1.7% to 36% [76], and rising with tuberculosis prevalence in general. As reported before in the present thesis, diabetes will make an increasingly important contribution to the tuberculosis epidemic. Experts suggest that tuberculosis control could be further improved by screening patients with known risk factors, including diabetes [77]. Screening for active tuberculosis in people with diabetes could hasten case detection, which could lead to earlier therapy and prevention of transmission. Furthermore, the administration of preventive tuberculosis therapy in tuberculosis-infected people with diabetes (identified by tuberculin test) could inhibit progression to tuberculosis. Therefore, to detect active tuberculosis as well as latent infection in diabetes in an early stage has significant public health relevance. Screening for tuberculosis in persons with diabetes still depends on chest X-ray and/or sputum smear now, and it is infeasible to offer this to all diabetes patients in the population. A sensitive, reliable and practical strategy is urgently needed. In agreement with other reported literature, we found that older age of $\geq$ 50 years, male gender and family history of diabetes are related with increased risk of tuberculosis. Further, vitamin D status in diabetes patients, together with underweight, as indicated in our study, may provide a new perspective. Therefore, we recommend that diabetes patients with the following

characteristics should be offered tuberculosis screening: poorly controlled blood glucose (HbA1c >7.0%); low body weight (BMI <18.5), especially severe emaciation (BMI <16); weight loss of >10% in the recent 2 months; persistent cough of more than one month; low serum 25(OH)D, especially if <10ng/mL. This should include chest X-ray and a tuberculin test [78; 79]. Male diabetes patients aged over 50 deserve extra attention.

Conversely, screening for diabetes in patients with tuberculosis could improve diabetes detection. Also, the rapidly increasing diabetes prevalence with a high proportion of undetected cases, being about 50% in developing countries, indicates the importance of screening of possible diabetes in newly admitted tuberculosis patients. Although this will not help detection of diabetes in the general population a lot, it may significantly benefit the clinical treatment of tuberculosis. As we have discussed, patients with concurrent tuberculosis and diabetes are prone to present atypical radiographic findings, severe signs and symptoms and higher treatment failure, relapse and death. When the project started, we found diabetes was not screened in local county level tuberculosis clinics in the setting of a rural area in China. Along with the implementation of the project, it has become a routine check item, and patients with diabetes have been identified at the beginning of the treatment. This has shown obvious improvement in the clinical course. We recommend that screening for diabetes should be routine clinical practice in TB patients.

Increase awareness of the double burden

Various strategies are needed to increase awareness of the double burden, especially in low- and middle- income countries of a large population. (1) Increase awareness of health providers: A stepwise training model was carried out in the present study and proven to be effective. Health providers, especially those working in primary health care centres should be paid

special attention. In China, village nurses play a core role in the practice of tuberculosis control in rural areas. They are usually the first choice for an individual with suspicious symptoms seeking for health service, and they are also responsible for the supervision of compliance of anti-tuberculosis therapy. However, due to low income level and a remote workplace, as we experienced in our study, young people with high level education are usually unwilling to take the job. This resulted in certain problems in village nurses such as older age, low education level, which may make them incapable to deal with the new challenge. (2) Increase awareness of the patients: During the implementation of the project, we introduced various strategies to increase the awareness in tuberculosis patients including a single sheet of knowledge paper, pictorial case report, handbook, etc to illustrate the key principle of the treatment, recommended balanced diet and healthy lifestyle to the patients. This proved to be useful. Also, in diabetes patients, the health education about the relationship between the diseases, the early signs and symptoms indicating tuberculosis should be carried out. (3) Increase awareness of the lay public: Family members of the patients, people in the community constitute the lay public. Posters, calendar, video compact disc, radio, etc. are good media for the health promotion.

The treatment of tuberculosis patients with concurrent diabetes
Diabetes is not only a risk factor for active tuberculosis, but also for poor tuberculosis treatment outcomes. Also, it may possibly be associated with increased risk of the development of multidrug-resistant tuberculosis (MDR-TB) [80] [81], although some studies reported different results [65]. As we have stated in the General Introduction, chronic hyperglycemia is associated with impaired immunity to *Mtb* in diabetes patients and may result in reduced efficacy of anti-tuberculosis treatment. The WHO recommended optimized glucose control as part of the management of combined patients

for improved tuberculosis outcomes [82]. Till now, widely accepted guidelines for the treatment of tuberculosis patients with concurrent diabetes have not been available. Recently, a group of expert clinicians provided treatment guidelines for drug-susceptible tuberculosis from a perspective of clinical therapy, with certain special recommendations for patients with combined diabetes. A total of 9 months of therapy is recommended, mainly based on the more severe presentation. Also, they suggested to measure drug concentrations in serum to gain insight into the adequacy of dosing and need for individual adjustments [83]. This is mainly based on the reported possibly suboptimal plasma levels of anti-mycobacterial antibiotics in diabetes patients. However, there is discrepancy at this stage [84] [85] and further study among patients with combined disease is required.

Besides these recommendations, we think vitamin D status should be monitored in tuberculosis patients given the high prevalence of (severe) vitamin D deficiency. Outdoor activity in order to ensure enough sun exposure, higher consumption of fish (especially sea fish), eggs, etc and vitamin D fortified items such as milk, butter are recommended. Vitamin D supplements may have a favourable adjunctive effect on clinical treatment in patients with concurrent diseases, but more confirmative studies are needed concerning the recommendation of vitamin D supplementation as a routine practice. The same holds for TB-only patients. Given the high prevalence of vitamin D deficiency, population supplementation may even prevent latent infection in areas with a heavy TB burden.

Future study

We observed higher prevalence of diabetes in active tuberculosis patients, and we found evidence that vitamin D deficiency plays a contributing role in

the risk of the combined diseases. Although causal relationships cannot with certainty be deduced from the present studies, we provided important etiological clues, and more longitudinal studies as well as confirmative trials concerning the role of vitamin D are needed.

Longitudinal study in patients with combined tuberculosis and diabetes
In recent years, several follow up studies among patients with combined tuberculosis and diabetes reported about aspects like the reversibility of diabetes, factors associated with mortality and change of circulating monocyte populations [51] [55] [57]. However, the number of subjects included was limited, and the follow-up duration was no longer than 6 months after the initiation of the treatment. Moreover, studies like that have never been carried out in China. During the implementation of this project, about 600 patients with concurrent tuberculosis and diabetes have been identified. Future longitudinal studies are needed: (1) to observe the long-term (1~2 years) treatment outcomes, including cure, relapse and death; (2) to collect information of possible markers of prognosis, including control of blood glucose, typical tuberculosis signs (cough, night sweating, dyspnea, etc), nutritional status (body weight, mid-upper arm circumference, etc); (3) to set up prognostic models to direct early prevention of unfavourable treatment outcomes in clinical practice.

Cohort study to verity the role of vitamin D deficiency in the double burden
In the present study, we have indicated that vitamin D deficiency may aggravate the increased risk of tuberculosis mediated by diabetes. However, due to the cross-sectional design and certain limitations in the vitamin D intervention study, we cannot draw a causal conclusion at this stage. Further prospective research comparing tuberculosis risk of diabetic subjects with

different vitamin D status (severely deficient, deficient and sufficient) is needed.

Confirmative trial of vitamin D in patients with concurrent tuberculosis and diabetes

We are the first to report a favourable adjunctive effect of vitamin D3 on the clinical manifestations in patients with concurrent tuberculosis and diabetes during intensive-phase treatment. Our results may indicate a new perspective for treatment. A trial with extra vitamin D in individuals without active disease may also show the potential for prevention of the combined diseases.

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Summary

Tuberculosis remains a major global health challenge, with an estimated 9 million incident cases worldwide each year, particularly in low-to-middle income countries. Although China has achieved a 50% reduction in pulmonary tuberculosis prevalence nationally since the 1990s, it still accounts for nearly 17% of the world's tuberculosis burden in 2016, with approximately 0.9 million new cases. At the same time, the prevalence of diabetes has increased especially rapidly over the last 10 years. The International Diabetes Foundation Diabetes Atlas estimated that in 2017 the number of persons with diabetes in China was 114 million, equaling to nearly a quarter of cases worldwide. Diabetes triples the risk of tuberculosis, resulting in more symptoms, relapses, treatment failures and deaths. Currently, 80% of global diabetes mellitus burden is in low-and middle-income countries. Six of them (India, China, Brazil, Indonesia, Pakistan and The Russian Federation) also have a high tuberculosis burden. An aggravation of the tuberculosis epidemic due to diabetes will pose a serious challenge for a relatively long period of time to come. In accordance, the WHO has recently identified type 2 diabetes as a neglected risk factor for the re-emergence of tuberculosis.

In recent years, the non-skeletal function of vitamin D has aroused intense interest. High prevalence of vitamin D deficiency was documented in many countries including China, representing a global health problem. Vitamin D deficiency is associated with increased risk of diabetes as well as the severity of the disease. At the same time, it is correlated with increased risk of tuberculosis independently of diabetes.

Therefore, we hypothesize that the double burden of diabetes and tuberculosis and its implications to public health in China are influenced by the high prevalence of vitamin D deficiency. If so, supplementation of vitamin D may open perspectives for prevention and even therapy. We carried out this study with the main purpose to investigate the epidemiology of co-occurrence of tuberculosis and diabetes and their risk factors and to elucidate the role of vitamin D deficiency in this interrelationship.

We carried out observational studies and an interventional trial in this thesis. First, a large epidemiological survey was carried out among 6382 newly diagnosed tuberculosis patients in a rural area in Linyi, Shandong, and among 6674 non-tuberculosis controls from the same community. Participants were screened for diabetes. The prevalence of diabetes in patients with active tuberculosis was 6.3% which was nearly 35% higher than in non-tuberculosis controls. Adjusted for confounders, tuberculosis patients had a slightly more than three times higher risk of having diabetes [OR(95%CI)=3.17(1.14-8.84)]. Risk factors for diabetes among tuberculosis patients were similar as observed in the general population in China (chapter 2). Further, 2280 tuberculosis patients were randomly selected from this study to analyze possible diagnosis delay and its risk factors. Hyperglycemia was associated with a nearly two-fold higher probability of patient delay of tuberculosis in the community (chapter 3).

Then, subjects diagnosed with tuberculosis only, concurrent tuberculosis and diabetes, diabetes only and normal controls were randomly selected for the analysis of serum vitamin D concentrations and its association with the risk of the diseases. A more than four-fold [OR(95%CI)=4.73(2.04-10.9)] and two-fold [OR(95%CI)=2.50(1.04-6.02)] increased risk of having tuberculosis only, and tuberculosis and diabetes combined was observed in the lowest quartile of serum 25(OH)D compared with the highest quartile (chapter 5). Subsequently a city level hospital-based study in Qingdao, Shandong was

carried out. A total of 461 tuberculosis patients were randomly selected to assess (severe) vitamin D deficiency and its determinants. A high prevalence of vitamin D deficiency [serum 25(OH)D<20 ng/ml] of nearly 80% was observed in tuberculosis patients, and severe baseline symptoms, low outdoor activity and low fish consumption were identified as the main determinants (chapter 4).

Then, we randomly selected a population of 299 tuberculosis patients from the same city level hospital that had 70% overlap with the population of chapter 4. As normal controls, we selected 91 individuals who underwent physical check-up from a general hospital in the same city. We investigated the correlations of vitamin D status with leptin and TNF-α. Besides its satiety regulatory function as a "hormone", leptin has also been recognized to have immune modulating properties in recent years. Vitamin D status was observed to be inversely associated with TNF-α, while positively associated with leptin, indicating a possible immune-modulatory mechanism of vitamin D in the pathogenesis of tuberculosis (chapter 6).

For an intervention trial, we were able to recruit 358 newly diagnosed pulmonary tuberculosis patients from the 461 patients reported in Chapter 4, aged ≥18 y, to study the effects of vitamin D3 supplementation (800IU/d) on tuberculosis clinical signs and symptoms and sputum conversion during the intensive-phase treatment. Compared those without diabetes, patients with concurrent diabetes had a higher baseline percentage of positive sputum (78.1% vs 64.7%), of cavities in CT scan (55.5% vs 38.5%) and had more severe clinical signs and symptoms. TBscore, a composite index of eleven signs and symptoms such as cough, hemoptysis, dyspnea, is a tool aiming to assess changes in the clinical status. The score was categorized into 3 classes, namely mild, moderate and severe. Improvement was defined as a change to a lower category. The improvement rates in vitamin D group

were 26.5% (4th week), 28.5% (6th week) and 31.0% (8th week), significantly higher than in the control group. Adjusted for confounders, i.e., age, gender, vitamin D supplementation was still independently associated with clinical improvement in patients with concurrent tuberculosis and diabetes from the 4th week during the intensive-phase treatment (chapter 7).

In this epidemiological study, the diagnosis of tuberculosis was made within the existing prevention and control system of the disease in China, following the criteria provided by the WHO. Also, diabetes was diagnosed according to WHO criteria for the classification of glucose tolerance based on fasting plasma glucose (FPG). Serum 25(OH)D concentrations were measured by using the method of radioimmunoassay. Major potential confounders such as BMI, age, smoking, drinking, physical activity, baseline cavity in CT scan were considered. We took various actions to ensure the reliability of the data in this study, including a standardized procedure of diabetes screening used in the multi-center investigation. Although residual confounding can never be completely ruled out, we think we minimized it as far as possible.

In conclusion, the risk of having diabetes in active tuberculosis patients was nearly three times higher compared to non-tuberculosis controls. In concurrent disease, diabetes frequently precedes tuberculosis, and age ≥ 50 years, male gender is associated with increased risk. Severe hypovitaminosis D was prevalent in tuberculosis patients, with a higher percentage in those with diabetes. Severe TBscore at admission, low outdoor activity and inadequate fish consumption were the main determinants. Vitamin D supplementation showed adjunctive effect in the therapy, i.e., improvement of tuberculosis clinical manifestations in this subpopulation. Regulation of immune activity related cytokines such as leptin and TNF-α, as well as modulation of glucose metabolism may be the possible mechanisms behind this adjunctive effect. Our result indicates an aggravating role of vitamin D deficiency in the double burden of tuberculosis and diabetes. The main

strength of the present study is the community based large-scale investigation which can assure the representativeness of the sample. In addition, a trial in patients with combined tuberculosis and diabetes has seldom been reported before. However, due to the cross-sectional design in chapter 2-6 and certain limitations in the vitamin D supplementation trial in chapter 7, we should be cautious in the interpretation of our study and a certain inference with respect to causality cannot be made at this stage.

Nevertheless, our study provides possible etiological clues for the combined occurrence of tuberculosis and diabetes and the possible role of vitamin D deficiency. There is a sufficient body of evidence in the literature, to which our present study adds, that diabetes increases the risk of active tuberculosis and influences the clinical course of tuberculosis. This may facilitate the transmission of tuberculosis in the community. Vitamin D deficiency may play an aggravating role in this process. Bidirectional screening for tuberculosis and diabetes, especially early detection of tuberculosis in diabetes patients, and increasing awareness of the double burden in various populations including the patients, health providers, and the lay public may have significant public health benefits. In addition, the possible adjunctive effects of vitamin D in patients with concurrent tuberculosis and diabetes may open a new perspective in clinical therapy, although we cannot recommend the supplementation as a routine practice at this stage. Also, supplementation may benefit tuberculosis patients in general. Moreover, extra vitamin D may benefit general population with especially in a situation of a heavy tuberculosis burden combined with prevalent vitamin D deficiency. We suggest future studies to address long-term treatment outcomes of patients with the combined diseases and to define certain markers to set up a prognostic model. Also, longitudinal

studies to verify the role of vitamin D deficiency in the double burden, and confirmative trials of the effect of vitamin D supplementation are needed.

总　　　结

　　结核病仍然是重大的公共卫生问题，全球每年估计有 900 万新发病例，主要在中低收入国家。尽管自 20 世纪 90 年代以来，我国肺结核患病率已经下降了近 50％，但结核负担仍然严重，2016 年新发病例约 90 万，占世界结核病负担近 17％。与此同时，糖尿病患病率在过去十年间增长迅速。据国际糖尿病基金会估计，2017 年中国糖尿病患者人数为 1.14 亿，相当于全球近四分之一的病例。糖尿病使结核病风险增加三倍，且临床症状更加严重、复发、治疗失败和死亡发生率增加。目前，全球 80％的糖尿病负担在低中收入国家，其中六个（印度，中国，巴西，印度尼西亚，巴基斯坦和俄罗斯）同时有高结核病负担。因此，由糖尿病导致的结核病流行将在相当长的一段时间对公共卫生构成严峻挑战。据此，世界卫生组织最近将 2 型糖尿病确定为结核病复燃的危险因素。

　　近年来，维生素 D 的骨骼外功能广受关注。包括中国在内的许多国家都存在维生素 D 缺乏，已成为一个全球性的健康问题。维生素 D 缺乏与糖尿病风险增加以及严重程度有关，与此同时，它与结核病的风险增加独立相关。因此，我们设想维生素 D 缺乏将加重糖尿病和结核病的双重负担，补充维生素 D 可能会为预防甚至治疗提供前景。因此，本研究的主要目的探讨结核病、糖尿病共患的流行病学特征、分析其危险因素，并阐明维生素 D 缺乏对糖尿病并发肺结核的影响。

　　我们开展了观察性研究和干预实验。首先，在山东临沂农村地区的 6382 名新诊断肺结核患者和来自同一社区的 6674 名未患结核对照人员中进行了大规模的流行病学调查，对所有参与者进行了糖尿病筛查。结果发现，活动性结核病患者的糖尿病患病率为 6.3％，比非结核病对照组高出近 35％。对混杂因素进行调整后，结核病患者患糖尿病的风险增

加三倍多[OR（95％CI）＝3.17（1.14-8.84）]。结核病患者中患糖尿病的危险因素与普通人群类似（第2章）。此外，我们从该研究中随机选取2280名结核病患者，分析了诊断延迟及其危险因素。发现高血糖者结核病诊断延迟的概率相比血糖正常者高出近两倍（第3章）。

随机选择单纯肺结核、糖尿病并发肺结核、单纯糖尿病和正常对照者，分析血清维生素D水平与疾病风险的关系。与血清25（OH）D最高四分位数者相比，最低四分位数者患单纯结核[OR（95％CI）＝4.73(2.04-10.9)]、糖尿病并发结核[OR（95％CI）＝2.50（1.04-6.02）]的风险分别增加约四倍、两倍（第5章）。随后我们开展了一项以市级结核专科医院为基础的研究。随机选择461名结核病患者评估维生素D缺乏情况及其影响因素。结果发现结核病患者中维生素D缺乏率[血清25（OH）D＜20 ng/mL]接近80％，严重的基线症状、户外活动水平低、鱼类消费量低是其主要危险因素（第4章）。我们随机选取了该医院的299名结核病患者（选取的患者与第4章的研究人群有近70％的重叠），并选择了91名在市级综合医院进行体检的人员作为正常对照。我们研究了维生素D水平与瘦素、TNF-α等的关系。除了作为激素调节"饱腹感"，近年来研究发现瘦素具有免疫调节功能。我们的结果发现血清维生素D水平与TNF-α呈负相关、与瘦素呈正相关，提示维生素D可能通过相关的免疫调节机制发挥作用（第6章）。

我们招募了符合纳入标准的新诊断肺结核患者进行干预试验，观察维生素D3补充（800IU／d）对结核病临床体征和症状、以及痰菌转阴的影响。与单纯肺结核比较，并发糖尿病患者基线菌阳率更高（78.1％ vs 64.7％）、CT片所示空洞更多（55.5％ vs 38.5％）、临床体征和症状更严重。 我们采用TBscore来评价临床症状，它是一种综合指数，包括咳嗽，咯血，呼吸困难等十一种体征和症状，是用于评估结核临床表现的工具。根据TBscore具体分值，将症状分为轻度、中度和重度，症状改变为较低级别被定义为"改善"。结果发现，维生素D组干预组的改善率分别为26.5％（第4周），28.5％（第6周）和31.0％（第8周），

显着高于对照组。调整年龄、性别等混杂因素后，维生素 D 补充仍然与强化期治疗期间第 4 周后患者的临床症状改善独立相关（第 7 章）。

本研究患者的诊断采用的是目前公认标准，结核病的诊断是根据我国现在实行的标准、糖尿病诊断根据 WHO 制定的基于空腹血糖（FPG）的葡萄糖耐量分类标准进行。血清 25（OH）D 浓度采用放射免疫法测量。我们考虑了研究中主要的潜在混杂因素，如 BMI，年龄，吸烟，饮酒，身体活动，基线空洞等。采取了各种措施确保研究中数据的可靠性，包括多中心调查中糖尿病筛查的标准化程序等。尽管流行病学研究中残余混杂不可能完全排除，但我们认为本研究已尽可能将其最小化。

总之，与非结核病对照相比，活动性结核病患者患糖尿病的风险几乎高出三倍。两病并发时，糖尿病常常发生在结核病之前，年龄≥50 岁、男性与发生风险增加有关。严重的维生素 D 缺乏在结核病患者中普遍存在，合并糖尿病时比例更高，入院时基线症状严重、户外活动量低和鱼类消费不足是其主要危险因素。维生素 D 补充剂在糖尿病并发结核的治疗中具有辅助作用，可促进结核病临床表现的改善。调节免疫活性相关细胞因子如瘦素、TNF-α，以及调节葡萄糖代谢可能是这种辅助效应背后的机制。我们的结果表明维生素 D 缺乏可加重结核病和糖尿病双重负担中。本研究的主要优势是基于社区的大规模调查，可以保证样本的代表性。此外，针对糖尿病并发结核患者的干预研究尚鲜见报道。然而，由于第 2-6 章中的横断面设计和第 7 章中维生素 D 补充试验的某些不足，我们在解释研究结果时应该谨慎，尤其是在进行因果关系推断时。

我们的研究为糖尿病并发肺结核、以及维生素 D 缺乏的可能作用提供了病因学线索。已有的文献中提供了两病双重负担的大量研究证据，本论文增加了新的证据，即糖尿病会增加活动性肺结核的风险并影响结核病的临床过程，这可能导致人群中结核病传播风险的增加，维生素 D 缺乏可在这个过程中起到加重作用。因此，结核病和糖尿病的双向筛查，特别是糖尿病患者结核病的早期检测，以及提高患者、医护人员以及社

区群众等对糖尿病、肺结核双重负担的认识，可对于结核的防控产生显著促进作用。此外，尽管目前我们尚没有充足证据提出补充维生素 D 作为常规手段，维生素 D 在糖尿病并发结核中的辅助作用可能为临床治疗开辟新的视角，此外，维生素 D 可能对单纯结核病患者也产生有益影响。同时，对于存在肺结核、糖尿病负担和维生素 D 缺乏普遍存在的情况下，额外的维生素 D 补充可能降低一般人群发生结核的风险。我们建议未来的研究应观察糖尿病并发结核的长期治疗结果、确定生物标志物以建立预后预测模型。此外，需要开展更多的纵向研究来验证维生素 D 缺乏在双重负担中的作用，以及补充维生素 D 效果的确证试验。

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About the Author

Curriculum Vitae

Qiuzhen Wang was born in Sichuan, China on 29th August, 1971. After she qualified in Medicine (School of Public Health) from Tongji Medical University, Wuhan, China in 1994, she practiced as a teacher in Qingdao University. During this period, she attended a Master degree program from 2000 to 2004 at the Institute of Human Nutrition of Qingdao University. From 2009 to 2013, she attended the PhD degree program at the Institute of Human Nutrition of Qingdao University. In October, 2013, she attended the second PhD degree program at the Division of Human Nutrition and Health, Wageningen University, The Netherlands. Currently, she is a professor in the Institute of Human Nutrition in Qingdao University. In recent years, she has focused on epidemiologic research of the double burden of tuberculosis and diabetes, and participated in the projects of "Screening and intervention of diabetes mellitus in patients with pulmonary tuberculosis in poverty regions in China (WDF 08-380)", and "the effect of vitamin D and retinol supplementation in patients with diabetes and pulmonary tuberculosis (NSFC)".

List of Publications
As first author

- **Wang Q,** Ma A, Han X, Zhao S, Cai J, Ma Y, Zhao J, Wang Y, Dong H, Zhao Z, Wei L, Yu T, Chen P, Schouten EG, Kok FJ, Kapur A. Prevalence of type 2 diabetes among newly detected pulmonary tuberculosis patients in

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- Cai J, Wang X, Ma A, **Wang Q**, Han X, Li Y. Factors associated with patient and provider delays for tuberculosis diagnosis and treatment in Asia: a systematic review and meta-analysis, Plos One, 10(3), 2015.

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Overview of completed training activities

Discipline specific activities

- "Food and Health" workshop, organized by China Nutrition Society, 2015

- "the double burden of TB and DM" workshop, organized by Institute of Human Nutrition, Qingdao University, 2016

- "Nutrition Science Book" editor workshop, organized by China Nutrition Society, 2018

- the 11th National Nutrition conference, organized by China Nutrition Society, 2013

- the 11th academic annual conference of Qingdao Nutrition Society , organized by Qingdao Nutrition Society , 2014

- the 12th academic annual conference of Qingdao Nutrition Society , organized by Qingdao Nutrition Society , 2015

- World congress on life sciences (WLSC2016), organized by China Association for Science and Technology,2016

- "nutrition in special population" conference, organized by China Nutrition Society, 2016

- Asia Pacific Clinical Nutrition Conference (APCCN2017), organized by Asia pacific clinical nutrition society, 2017

- "National standards for food for the elderly" symposium, organized by China Nutrition Society, 2018

- Academic conference on special nutrition (11thCNSSN, 2018), organized

by China Nutrition Society, 2018

General courses

- Basic Toxicology, organized by Qingdao University

- Literature research, organized by Qingdao University

- Medical Statistics, organized by public health department, Qingdao University

Optionals

- Preparation of research proposal, organized by the research group in Qingdao University

- Staff Seminars, organized by Division of Human Nutrition, Wageningen University

- Weekly group meetings, organized by the research group in Qingdao University