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Original Article

Profile of glycated-hemoglobin, antioxidant vitamin and cytokine levels in pulmonary tuberculosis patients: A cross sectional study at Pulmonary Diseases Center Semarang City, Indonesia

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ABSTRACT

Background: Uncontrolled blood glucose, which marked by high level of HbA1c, increases risk of pulmonary TB because of cellular immunity dysfunction. This study aimed to analyze profile of glycated hemoglobin, antioxidant vitamins status and cytokines levels in active pulmonary TB patients.

Methods: This was a cross sectional study, conducted at Pulmonary Diseases Center Semarang City, Indonesia. Study subject consisted of 62 pulmonary TB patients, diagnosed with positive acid fast bacilli and chest X-ray. ELISA was used to measure IFN-γ and IL-12. Status of antioxidant vitamins was determined by concentration of vitamin A and E using HPLC. Blood glucose control was determined by HbA1c concentration (HbA1c ≥7% is considered as uncontrolled).

Results: A significant difference of age between pulmonary tuberculosis patients with normal and uncontrolled blood glucose ($p = 0.000$) was showed, while all other characteristics (sex, education, occupation) did not differ with $p = 0.050$, 0.280, 0.380 respectively. Mean HbA1c was 7.25 ± 2.70%. Prevalence of uncontrolled glucose among pulmonary TB patients was 29%. Levels of IFN-γ and IL-12 did not differ according to HbA1c concentration ($p = 0.159$ and $p = 0.965$ respectively). Pulmonary tuberculosis patients with uncontrolled blood glucose has higher vitamin E ($p = 0.006$), while vitamin A did not differ significantly ($p = 0.478$).

Conclusions: This study supports the importance of performing diabetes screening among pulmonary TB patients. Further study needs to be done to determine the feasibility of TB-DM co-management.

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At a glance commentary

Scientific background on the subject

Indonesia has a second largest burden of tuberculosis globally, and had 10 million cases of diabetes in 2015. Relationship of tuberculosis and diabetes is more prominent in developing countries, where TB is endemic and the burden of diabetes mellitus is increasing. Early detection uncontrolled glucose and antioxidant vitamins in pulmonary tuberculosis patients may give benefit to control the diseases.

What this study adds to the field

Almost one third of PTB patients had HbA1c levels >7%. The group of pulmonary tuberculosis with high HbA1c levels had similar levels of vitamin A, IFNγ, and IL-12. On the other hand, vitamin E levels was higher in HbA1c > 7. Management of co-morbidities need to prioritize at risk group. This study supports the importance of performing blood glucose screening among pulmonary tuberculosis patients.

Glycated hemoglobin, commonly known as HbA1c, reflects an average blood glucose levels over a period of weeks or months. The International Expert Committee has recommended HbA1c for diabetes diagnosis [1]. Worldwide, the International Diabetes Federation (IDF) predicts that the number of people with diabetes will rise by 55% in the next 20 years [2]. The current pandemic of type 2 diabetes mellitus is accelerating in a world where approximately one third of the population is latently infected with *Mycobacterium tuberculosis* [3]. Diabetes patients have 2–5 times higher risk to develop tuberculosis compared to non-diabetes [4,5]. Once infected, diabetes patients need longer treatment and are at risk of drug resistance [6] and treatment failure [7]. Furthermore, a study in Indonesia revealed pulmonary tuberculosis patients with co-existence of diabetes had more clinical signs compared to non-diabetes [8].

Uncontrolled blood glucose, which marked by high level of glycated hemoglobin (HbA1c) [9], is one of TB risk factors [10]. Risk of active tuberculosis development in population with diabetes [4,11,12] may relate to lower antimicrobial peptide gene expression [13]. The lower antimicrobial peptide gene expression may also increase the risk of tuberculosis reactivation [13,14] and modifies clinical sign of pulmonary tuberculosis [15]. Diabetes patients are also prone to pulmonary TB infection due to phagocytosis [16] and cellular immunity [17] dysfunctions, the two main immune responses to *M. tuberculosis* [18].

Pulmonary tuberculosis and diabetes are often associated with malnutrition. The link between nutritional status and tuberculosis has long been appreciated, but remains an emerging area of study that has focused on investigations of related biomarkers and nutrient supplementation trials [19]. Pulmonary tuberculosis patients frequently suffer from deficiencies in antioxidant micronutrients, such as vitamins A and E. Both are fundamental vitamins to the integrity of the immune response, especially the host's immune response against *M. tuberculosis*. Furthermore, vitamins play an important role in glucose metabolism [20]. Vitamins also related with active pulmonary tuberculosis. A previous study showed that the levels of vitamin C and vitamin E were significantly decreased in *M. tuberculosis* patients when compared to the healthy controls [21].

Both diabetes and pulmonary tuberculosis patients are usually underdiagnosed, or delayed diagnosed [22]. Early detection of diabetes through blood glucose indicator in pulmonary tuberculosis patients, along with the underlying factors might play role in co-management of the diseases in question. In this study, we analyzed the cytokines (IFN-γ and IL-12), antioxidant vitamin (vitamin A and E), and blood glucose control (HBA1c) in active pulmonary TB patients.

Methods

Study subject

Subject was pulmonary tuberculosis patients, who sought treatment at Pulmonary Health Center (called BKPM = *Balai Kesehatan Paru Masyarakat*) Semarang City, Indonesia [Fig. 1]. Patients were selected by pulmonary tuberculosis symptoms as follow: persistent cough for more than 3 weeks, produce sputum, and loss weight. Two same-day sputum specimens were collected and examined for sputum smear microscopy by Ziehl Neelsen staining, and detected to have acid fast bacilli on sputum examination [23]. Patients also underwent chest X-ray to examine the existence of tuberculosis cavities. HIV serology test was done to all pulmonary tuberculosis suspects for exclusion criteria.

Eligible subjects were selected consecutively during June 11–August 23, 2014. During the course of study, there were 85 patients diagnosed as pulmonary tuberculosis, 23 of them either refused to join the study or experienced difficulties during blood collection. Overall, there were 62 pulmonary tuberculosis patients became our study subject. All subjects agreed to join the study by signing written informed consent. Ethical clearance was obtained from the Commission of Ethics of Medical and Public Health Research, Faculty of Public Health, Diponegoro University (number: 298/EC/FKM/2013).

Data collection

Variables in this study were pulmonary tuberculosis occurrence, body mass index (BMI), IFN-γ, IL-12, vitamin A, vitamin E, HbA1c levels, characteristics (age, sex, education, occupation), and history of diseases history of diseases (diabetes, high blood pressure, hypercholesterolemia). Pulmonary TB referred to patients with *M. tuberculosis* infection, which is characterized by detection of acid fast bacilli in sputum using Ziehl Neelsen examination and chest X-ray. Body mass index was derived from ratio of body weight in kilogram to body height in centimeter. Education was categorized as basic

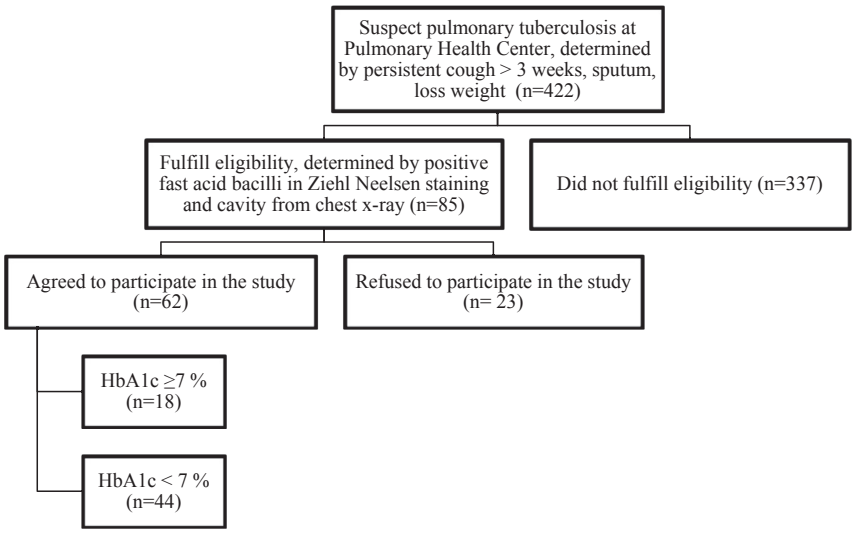

Fig. 1 – Flow diagram of study subject participation.

(elementary to junior high school) and high education (senior high school and college). Occupation was categorized as employed and unemployed.

IFN-γ and IL-12 were examined using Quantikine HS ELISA kits and read in 450 nm. Vitamin A was measured in the form of retinol serum concentration indicator, using HPLC examination and stated in μg/L. Vitamin A below 200 μg/L is considered as deficiency. Vitamin E was measured in the form of alpha tocopherol, which preferentially absorbed and accumulated in human. The measurement also used HPLC, which stated in mg/L, and value <3 mg/L is considered as deficiency. HbA1c levels was measured as mean of blood glucose concentration in form of glycated hemoglobin using HPLC examination in %. HbA1c indicates blood glucose control within previous three months, and is considered as diabetes if its exceeds recommendation borderline of 7%. All laboratory examination was conducted at R&E Unit, Prodia Laboratory, Indonesia.

Data analysis

Distribution of data was determined by Kolmogorov–Smirnov test. Independent t-test was used to analyze data the difference of IFN-γ and IL-12, vitamin A and vitamin E concentration in pulmonary tuberculosis patients with uncontrolled and normal blood glucose levels. The difference of characteristics was analyzed with chi-square (education, occupation, history of diseases) and independent t-test (age).

Results

Our study showed 18 out of 62 (29.0%) pulmonary tuberculosis patients had uncontrolled blood glucose, which was indicated by HbA1c levels >7% [Table 1]. There was a significant difference between age ($p = 0.000$) and diabetes history ($p = 0.000$) of pulmonary tuberculosis patients with high and normal glycated hemoglobin levels, while all other variables did not differ significantly from each other [Table 2]. Mean HbA1c levels of all subjects was 7.25%. Mean of vitamin A from all subjects (307.29 μg/L) were considered as normal (200–800 μg/L). Mean concentration of vitamin E of all subjects (14.94 mg/L) was slightly higher than normal (8–14 mg/L) [Table 3].

Pulmonary tuberculosis patients with high HbA1c levels had similar vitamin A, IFN-γ and IL-12 with those with normal HbA1c. Statistical analysis also proved the vitamin A, IFN-γ and IL-12 did not differ significantly between both groups ($p = 0.159$, 0.965 and 0.478 respectively). On the other hand, pulmonary tuberculosis patients with HbA1c ≥7% had higher vitamin E concentration (16.78 ± 3.49 mg/L) compared to the normal group (14.18 ± 3.17 mg/L). The difference of vitamin E levels was statistically significant ($p = 0.006$).

Discussion

Our study showed most of pulmonary tuberculosis patients were male, i.e. 67.7% (in all subjects), 77.8% (in normal HbA1c group) and 63.6% (in high HbA1c group). This result is in accordance with previous descriptive epidemiology studies in Indonesia [24] and worldwide [25–27]. Men seem to be more affected than women, with a male/female ratio of 1.9 ± 0.6 for the worldwide case notification rate [28]. The global tuberculosis epidemic is also characterized by significant differences in prevalence between men and women. Rates of tuberculosis are much higher among men than women in large areas of the world, and it has been extensively documented [29].

Table 1 – Prevalence of blood glucose control among pulmonary tuberculosis patients.

HbA1c levels	n = 62	%
≥7%	18	29.0
<7%	44	71.0

Table 2 – Characteristics of pulmonary tuberculosis patients.

Variables	All subject (n = 62)	HbA1c ≥7% (n = 18)	HbA1c <7% (n = 44)	*p*
Age (years)	40.06 ± 15.46	52.72 ± 9.27	34.89 ± 14.51	0.000[a]
BMI (kg/m^2)	19.19 ± 3.22	20.44 ± 3.84	18.68 ± 2.83	0.050[a]
Sex: male	67.7%	63.6%	77.8%	0.280[b]
Education: basic	58.1%	54.5%	66.7%	0.380[b]
Occupation: unemployed	27.5%	31.8%	16.7%	0.225[b]
History of diabetes	21.0%	66.7%	2.3%	0.000[b]
History of high blood pressure	12.9%	11.1%	13.6%	0.788[b]
History of hypercholesterolemia	6.5%	16.7%	2.3%	0.127[b]

[a] Independent t-test.
[b] Chi-square test.

Age of subjects was mostly in productive ages, with most of them were 17–35 years of old. Descriptive epidemiology studies have previously found the same pattern [30,31]. Recent study revealed polymorphism in mannose lectin (MBL) is the factor affected high case of tuberculosis in productive ages [32]. When associated with HbA1c levels, our result indicated mean age of pulmonary tuberculosis patients with HbA1c ≥7% (52.72 ± 9.27 years) was higher than those with normal HbA1c (34.89 ± 14.51 years). A previous study showed that tuberculosis patients with diabetes condition tends to found in older age [33]. We suggest health institutions with tuberculosis service to pay attention on patients with higher risk of co-morbidities such as diabetes. Identification of the high risk groups allows the health institutions to prioritize age groups that needs to gain more attention in management of co-morbidities, in this case tuberculosis and diabetes.

Prevalence of pulmonary tuberculosis patients who have uncontrolled blood glucose with HbA1c ≥7% was 29.0%. The prevalence is in accordance with previous report that one third of the diabetes population is latently infected with *M. tuberculosis* [3]. Our study revealed most of subject with HbA1c ≥7% had history of diabetes (12 out of 18 subjects). This needs attention since impaired glucose tolerance is a significant risk factor for developing diabetes [34]. A previous study showed tuberculosis subjects had more comorbidities than population control subjects, including diabetes [25]. The relationship between diabetes and tuberculosis is bi-directional [12]. This study supports the importance of performing diabetes screening among pulmonary TB patients. Further study needs to be done to determine the feasibility and cost-effectiveness of co-management of TB-DM.

Our study showed a higher concentration of vitamin E in pulmonary tuberculosis patients with HbA1c ≥7%. Vitamin E is the most important lipid phase chain breaking antioxidants, and is central to the prevention of lipid peroxidation [21]. Tuberculosis patients usually demonstrate high levels of lipid peroxidase and low concentration of plasma vitamin E [35]. Lipid peroxidation has also been considered as an early marker for diabetes [36].

Previous studies observed that vitamin E was significantly depleted in tuberculosis patients when compared with healthy individuals [21,37]. Administration of micronutrients, including vitamin E, has been shown to enhance antioxidant status [35] and accelerate tuberculosis healing [38]. Vitamin E presumably will also give benefit to tuberculosis patients with diabetes. An *in vivo* study revealed supplementation with vitamin E significantly reduced glycemia and glycated hemoglobin (HbA1c) values in diabetic rats [39]. A similar result was also shown from an experiment study in diabetic patients, which demonstrated that administration of vitamin E decreased HbA1c levels in patients with inadequate glycemic control (HbA1c ≥8%) and low vitamin E levels [40].

Our study found vitamin A did not differ between pulmonary tuberculosis patients with normal and inadequate blood glucose control. The result was in contrary with a previous study that showed tuberculosis and diabetes often coincide with micronutrient deficiency, including vitamin A [20]. Several studies worldwide observed low level of vitamin A in tuberculosis patients [41–43]. However, the effect of vitamin A supplementation on tuberculosis patients in Indonesia varies according to nutritional status of the patients. In patients with good nutritional status, supplementation of vitamin A resulted in earlier sputum smear conversion [44]. On the contrary, vitamin A supplementation failed to reduce sputum conversion time in severely malnourished patients [45].

Various studies have shown the benefits of both vitamin E and vitamin A in tuberculosis patients, or in diabetes patients separately. Yet to the best of our knowledge, there has been no

Table 3 – Status of HbA1c, cytokines and vitamins of pulmonary tuberculosis patients.

Variables	Mean ± SD (n = 62)			Min–max	*p*[a]
HbA1c (%)	7.25 ± 2.70			4.4–14.4	
		HbA1c ≥7%	HbA1c <7%		
IFN-γ	19.14 ± 13.31	22.76 ± 12.02	17.63 ± 13.66	0.82–48.5	0.159
IL-12	2.58 ± 1.63	2.57 ± 1.57	2.58 ± 1.67	0.5–8.42	0.965
Vitamin A (μg/L)	307.29 ± 149.55	328.78 ± 184.09	298.50 ± 134.37	93–883	0.478
Vitamin E (mg/L)	14.94 ± 3.45	16.78 ± 3,49	14.18 ± 3.17	8–25	0.006

[a] Independent t-test.

research to study the effect of vitamin E or vitamin A in tuberculosis with inadequate glycemic control (high HbA1c). Therefore, comparison with existing literature could not be made. We found Wang et al. (2013) conducted a randomized controlled trial to determine the effect of vitamin A in the form of retinol on treatment of active pulmonary tuberculosis [20], but the result has not been published.

Our study also did not found difference of IL-12 and IFN-γ in pulmonary tuberculosis patients with normal or HbA1c ≥7%. This is possibly due to both tuberculosis and uncontrolled glucose contribute in decreasing IL-12 and IFN-γ, thus resulting in similar cytokines profile. A longitudinal study by Tsukaguchi showed tuberculosis patients whose blood glucose was under control in six months will undergo an improvement in IFN-γ production. On the contrary, IFN-γ levels in tuberculosis patient with high or uncontrolled blood glucose remained low. This means production of IFN-γ is more related to blood glucose status than to history of diabetes [46].

T cell-mediated immune responses are important in the host control of *M. tuberculosis* infection. This is evident from the increased risk of tuberculosis in individuals with deficiencies in their IFNγ and IL-12; which promotes TH1 cell differentiation signaling pathways [47]. When associated with blood glucose, the results from previous studies were contradictory. Gomez (2013) showed monocytes from diabetic patients express the decrease of IL-12 and IFN-γ secretion *in vitro*, but the relation was affected by interaction with bacteria [48]. Chu (2015) revealed glucose increases IL-12 production in stimulated PBMCs of diabetes patients through increased IL-12 gene expression [49]. An experiment in streptozotocin-induced mouse showed that *M. tuberculosis* infection causes IL-12 and IFN-γ to decrease [18]. A metabolomic study to determine whether there is a direct functional connection between serum abundance of metabolites and of immune mediators found significantly elevated serum concentrations of IFN-γ and soluble alpha chain of the IL-2 receptor alpha in TB active patients as compared to the two healthy groups [50].

There were several limitation of this study. The diagnosis of pulmonary tuberculosis did not include culture. This was related to tuberculosis program in Indonesia that only uses fast acid bacilli detection and X-ray. In addition, history of diabetes was only determined from interview with subjects and medical record if any, not by fasting and postprandial glucose serum levels. The level of nonresponse is also a concern. Proportion of eligible subjects who refused to participate in this study was 27% (23 out of 85 eligible patients). Therefore, generalization must be taken with caution due to sample size. Besides, this study used cross sectional design, which cause difficulty to establish relation between outcome and exposure of long duration like pulmonary tuberculosis and high blood glucose. A longitudinal design should be considered to investigate the role of underlying factors of pulmonary tuberculosis in conjunction with HbA1c levels.

Conclusions

Nearly one third of pulmonary tuberculosis patients in this study (29.0%) contracted with uncontrolled blood glucose, which was indicated by HbA1c levels >7%. Overall, mean HbA1c was 7.25%. Pulmonary tuberculosis patients with HbA1c ≥7% had higher age, BMI, IFN-γ, vitamin A and vitamin E levels. However, only vitamin E that significantly difference in pulmonary tuberculosis patients according to HbA1c levels.

Identification of high risk groups allows the health institutions to prioritize age groups that needs to gain more attention in management of co-morbidities, in this case tuberculosis and diabetes. This study supports the importance of performing diabetes screening among pulmonary TB patients. Further study needs to be done to determine the feasibility and cost-effectiveness of co-management of TB-DM.

Conflicts of interest

We declare that we have no conflict of interest.

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