

# Vitamin D and Its Association with Tumor Necrosis Factor-alpha Level in Active Tuberculosis Patients in North Sumatera, Indonesia

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Abstract: Background: *Mycobacterium tuberculosis* is one of bacteria that caused tuberculosis disease. This disease related to macro and micronutrient deficiency, also to inflammation process. One of deficiency micronutrient is vitamin D deficiency, host cell response in including deficiency vitamin D, this would affect tumor necrosis factor (TNF)-alpha level. Objective: We sought to determine whether there is an association between vitamin D status and TNF-alpha status in active tuberculosis patients. Design: We conducted a cross-sectional study of 25 man and women aged 18-60 years with active tuberculosis in North Sumatera, Indonesia. Parameters were 25(OH)D, TNF-alpha serum level, biomolecular parameters such as albumin and hs-CRP also assessed. The association was analysis using chi-square or fischer test. Results: the mean of study subjects age were 37.2±14.9 years old, there were 81.2% subjects categorized into vitamin D deficiency-insufficiency and 18.8% categorized into vitamin D sufficiency. All of the subjects had high TNF alpha level, There is an association between vitamin D and TNF alpha serum level. Conclusions: based on this result, there was an association between vitamin D and TNF-alpha serum level inactive tuberculosis.

## 1 INTRODUCTION

Tuberculosis had become a global health problem in developed countries, including Indonesia, which have economic and social problem. Previous research reported higher macro and micronutrient deficiency, including vitamin D deficiency in normal and active tuberculosis patient who lived in abundant sunlight exposure (Sari et al., 2017a, 2017b). Vitamin D deficiency related to higher inflammatory effect, host cell respons with tuberculosis infecton showed higher proinflammatory agent including TNF alpha, interleukin (IL)-1, IL-2, IL-8, interferon (IFN) gamma, and granulocyte-macrophage colony stimulating factor. The association between vitamin D deficiency and TNF alpha in tuberculosis remain poorly understood. Previous research focused on TNF alpha, because of higher inflammatory effect of this cytokine. One of the theories is vitamin D would inhibit the growth of *M. tuberculosis* in the macrophages (Crowle et al., 1987; Rook et al., 1986). Vitamin D also promote anti-*M. tuberculosis* responses through many mechanisms in the macrophage. The mediators

that involved in the macrophage were nitrit oxide (NO) (Rockett et al., 1998), NADPH oxidase (Sly et al., 2003; Sly et al., 2001), cathelicidin (P. T. Liu et al., 2006; Philip T et al., 2007; Yuk et al., 2009), and autophagy (Yuk et al., 2009) mechanisms. The vitamin D action that would inhibit progression of tuberculosis is impaired *M. tuberculosis* activity in the macrophage and inhibit proinflammatory signalling, including TNF alpha signalling.

The aims of this study were to determine whether there is an association between vitamin D status and TNF-alpha status in active tuberculosis patients in Medan, North Sumatera, Indonesia.

## 2 METHOD

We conducted a cross-sectional study of 25 man and women aged 18-60 years in North Sumatera, Indonesia April to July 2018, during the dry season (dry season in Indonesia is between April and October, higher sunlight exposure, lower rainy season). The location of recruitment was in Sumatera Island (Medan, North

Sumatera, Indonesia) with latitude: 3.67 N and longitude 97.55 E, average temperature:  $\pm 32^{\circ}\text{C}$  ( $90^{\circ}\text{F}$ ). This study was carried out after ethical approval was obtained from the Health Research Ethics Committee of Sumatera Utara University Medical School (No. 96/TGL/KEPK FK USU-RSUP HAM/2018) and all participants were given written informed consent to the study procedures. and all participants were given written informed consent to the study procedures.

### 2.1 Study Participants

The subjects of this study consisted tuberculosis patients in two community health centers with the higher tuberculosis prevalence in Medan, North Sumatera, Indonesia. Two community health centers were in Puskesmas Teladan and Puskesmas Amplas. There were man and women with various occupations, and taken purposively, there were 25 subjects that include in this study. The inclusion criteria were active tuberculosis patients within the range of 18-60 years old. Exclusion criteria were subjects with history of diabetes mellitus, myocardial infarction, renal or liver dysfunction. In addition to those exclusion criterias, subjects who were HIV, pregnant and lactating were also excluded.

### 2.2 Laboratory Analysis

We measured 25(OH)D serum concentration by chemiluminescent immunoassay (CLIA) technology (Diasorin, Stillwater, MN), measures were between 4.0 and 150 ng/mL. The lowest value was 4.0 ng/mL which is based on an inter-assay precision 3.90% CV. Reference range were <20 ng/mL categorized deficiency, 20-30ng/mL (insufficiency), 30-100 ng/mL (sufficiency) (Sharma & Meena, 2017). To convert ng/mL to nmol/L is multiply with 2.496. We measured albumin serum using architect C800 with colorimetry method and to measured hs-CRP, we also use architect C800 with different method which are turbidimetry/immunoturbidimetry. Tumor necrosis factor alpha analysis using ELISA (Enzyme-Linked Immunosorbent Assay) using Quantikine ELISA Human TNF alpha, R&D, USA.

### 2.3 Statistical Analysis

Continuous variables were expressed as continuous variables as means $\pm$ SDs. Categorical variables were expressed as percentage proportions, using chi-square to expressed significance difference between two groups, and Fischer test if the data did not met the

criterias. The *p* values <0.05 were considered statistically significant. We used SPSS program (version 11.5; SPSS Inc, Chicago, IL) to perform the analysis.

## 3 RESULTS AND DISCUSSIONS

The results will be discussed in 4 subsections, they are clinical characteristic of study participants, vitamin D and biomolecular parameters, and tumor necrosis factor alpha serum level and its association.

### 3.1 Clinical Characteristic of Study Participants

The aim of this study was to determine whether there is association between 25(OH)D with TNF alpha serum in active tuberculosis patients in tropical country. In this study showed that the higher prevalence of age classification was in 31-40 years (64%, mean $\pm$ SD: 35.3 $\pm$ 5.9 years), most of the subjects were male (80%), and based on BCG scar, the subjects were had clear BCG scar (Table 1). Based on this result, BCG scar seemed had no effect on tuberculosis incidence, probably because of long state of malnutrition or unsuccessful immunization. Protein and fat had influence in nutrition status in tuberculosis patient. Low protein intake would lower immunity cell activity, this would affect *Mycobacterium tuberculosis* infection in malnutrition patients.

Table 1. Clinical characteristics

Variables	n(%)
Distribution by severity class/TB score	
Class I	25 (100)
Class II	-
Class III	-
BCG scar	
Clear	17 (68)
No scar	3 (12)
Dubious	5 (20)

Abbreviations:

SD: Standart Deviation

BCG: Bacille Calmette-Guérin

### 3.2 Vitamin D and Biomolecular Parameters

Based on this study, most of the active tuberculosis patients had deficiency-insufficiency vitamin D (90.2%), the effects of vitamin D to tuberculosis progression through vitamin D receptor (VDR). This VDR activity, which is the signaling would induces a series of antimicrobial responses such as induction of autophagy, phagolysosomal fusion, release, and activation of the antimicrobial peptide cathelicidin, and killing of intracellular *Mycobacterium tuberculosis* (Gibney et al., 2008; Yoon et al., 2013). There also many research about VDR gene, especially polymorphism of VDR gene which were TaqI and FokI. The patient with polymorphism would resulting lower vitamin D serum level and higher infection of *Mycobacterium tuberculosis*. Previous studies also report about polymorphism of TNF alpha polymorphism, that would resulting ineffective of TNF alpha activity. Low albumin serum showed lower immunity cell activity, interestingly in this study showed most of the subject had normal albumin serum level, but yet still had active inflammation. This would need another explanation to this phenomenon.

Table 2. Serum levels of 25-hydroxyvitamin D and biomolecular parameters

Parameters	Mean±SD; n(%)
25-hydroxyvitamin D serum levels (ng/mL)#	21.8±7.6
Vitamin D status, n (%)	
Deficiency-insufficiency	22 (88)
Sufficiency	3 (22)
Albumin (g/dL)	3.8±0.8
Low	6(24)
Normal	19(76)
High	-
hs-CRP (mg/dL)	49.1±15.7
Low inflammation	-
Active inflammation	25(100)

Continues variable: mean ± SD; categorical variable: n (%); SD=standard deviation

### 3.3 Tumor Necrosis Factor-alpha Serum Level

Tumor Necrosis Factor-alpha (TNF-alpha) is a 17.5kDa, 157 amino acid protien that is a potent lymphoid factor, which produced cytotoxic effects on a wide range of tumor cells and other target cells. In the cell, TNF alpha is a pro-inflammatory and the primary mediator of immune regulation. The biosynthesis of TNF alpha is a major secreted factor in activated infected cells besides other proinflammatory cytokines (P. D. Davies, Brown, & Woodhead, 1985; P. D. O. Davies, 1985; Wilkinson et al., 2000). In tuberculosis infection, human macrophages exposed to M. tuberculosis demonstrated a robust release of TNF-alpha degradation, including other inflammation parameter such as NF-kappa-B nuclear translocation and other interleukin (P. D. Davies et al., 1985; P. D. O. Davies, 1985).

In this study showed as presented in **Table 3**, higher TNF alpha serum level, this probably because of status of the study subjects. All of the study subject were new cases and active tuberculosis. They also had lower nutrition status, macro and micronutrient deficiency including lower protein status and vitamin D deficiency.

Table 3. TNF alpha serum levels

Parameters	Mean±SD; n (%)
Tumor necrosis factor alpha serum levels (pg/mL)#	167.15±45.5
TNF alpha categorized, n (%)	
Low	-
Normal	-
High	25 (100)

Continues variable: mean ± SD; categorical variable: n (%); SD=standard deviation

### 3.4 Association between Vitamin D and TNF-alpha

There was an association between vitamin D status and TNF alpha status in active tuberculosis patient, this study reported that when there was a vitamin D deficiency and insufficiency, it was an association with higher TNF alpha serum level (**Table 4**). Other previous results showed there was an association also between vitamin D level and TNF alpha serum level (Anandaiah et al., 2013).

Table 4. The analysis of vitamin D and TNF alpha status

		TNF alpha status, n(%)		<i>p</i>
		Nor-mal	High	
Vitamin D status, n(%)	Deficiency-insufficiency	22 (88)	25 (100)	0.02*
	Sufficiency	3 (22)	0	
Total		5 (100)	25 (100)	

Using Fischer test, \* significant

Limitation of this study was we have not yet get the information about genetic polymorphism of vitamin D receptor gene and TNF alpha gene. We also had a limitation in the lower sample size.

## 4 CONCLUSIONS

Based on this result, there was an association between vitamin D and TNF-alpha serum level in active tuberculosis.

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