Research Article

Effects of Probiotics and Vitamin B Supplementation on IFN-γ and IL-12 Levels During Intensive Phase Treatment of Tuberculosis

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ABSTRACT

Tuberculosis (TB) is an acute infectious disease that primarily affects the lungs. Probiotics supplementation can increase the number and activity of NK cell in peripheral blood by modulation of interleukin-12 (IL-12), thus increasing interfenon-γ (IFN-γ) production by T-helper cells type 1 (Th1) response. Vitamin B_1 acts on macrophages and affects neutrophil motility. Vitamin B_6 is associated with the release of cytokines and the responsiveness of NK cells, while vitamin B_{12} affects to lymphocytes, Tcell proliferation, $CD4^+$ ratios, and NK cell activity. This study aim to analyze the effects of probiotics and vitamin B_1, B_6, B_{12} supplementation on IFN-γ and IL-12 levels during intensive phase of antituberculosis treatment. The study was pre-post test randomised control by time series. The control group was TB patients with standard therapy of antituberculosis and vitamin B6, while the intervention group was TB patients receiving therapy plus once daily probiotics and vitamin B_1, B_6, B_{12} supplementation during the intensive phase. Blood samples were withdrawn at baseline, one month, and two months after therapy to measure plasma IFN-γ and IL-12 levels using the ELISA method. Twenty two patients were divided equally into two groups. There was a tendency to greater increase of IFN-γ in the first month of the intervention group, followed by a significant decline after two-month therapy ($p < 0.05$). In both groups there was a rise in IL-12 levels after one month followed by a decrease in the second month ($p > 0.05$). However, the percentage was higher in the supplementation group. Addition of probiotics and vitamins B_1, B_6, B_{12} could improve immune response through IL-12 and IFN-γ modulation during intensive phase therapy.

Keywords: Interleukin-12, Interferon-γ, Probiotics, Tuberculosis, Vitamin B

INTRODUCTION

Tuberculosis (TB) is an acute infectious disease caused by *Mycobacterium tuberculosis* (Mtb). TB can be transmitted from one individual to another through airbone droplets. Globally, TB is the leading cause of death from a single infectious agent, killing approximately 1.67 million persons in 2016. At least one quarter of persons worldwide, approximately 1.7 billion people, are infected with Mtb. Among these infected persons, approximately 10% or 170 million persons globally, will be expected to develop TB during their lifetimes (WHO, 2018). According to the recent report, there are new high burden countries lists for the period of 2016–2020 (TB, Multidrug-resistant TB (MDR-TB) and TB/ HIV), and Indonesia is among the 20 top countries with the incident of 10.000 per year for TB, and 1,000 per year for TB/HIV (WHO, 2017).

The body's defense system for the intracellular bacterial infections which have important role are macrophages and Th1 cells. Those act as a specific mediator in destroying Mtb. Fagocytic cells that influx bacteria and become apoptosis will be uptaken by lung dendritic cell then transported to lymphe. This is the stimulate adaptive immune phase, T-helper tipe 1 (Th1) stimulates the cytokine secretion (Etna, 2014). Cytokines that have important contribution to TB infection include IFN-γ, TNF-α, IL-12, IL-17, IL-10, and IL-6.

In turn cytokine will stimulate neutrophils and activate macrophages, form granulomas, and form antimicrobial agents such as oxygen and nitrogen reactive to inhibit Mtb (Adrian *et al*, 2015).

Probiotics can modulate the immune system through the activity of epithel cells and immune cell receptors. In respiratory tract infections, there is an increase in the activity of fagocytes from alveolar macrophages after the administration of probiotics, thus increasing the activity of NK cells in the IFN-γ production (Forsythe, *et al.*, 2013; Mortaz, *et al.*, 2013).

Some vitamins can affect to body's immunity, including cell-mediated immunity, humoral immunity, and phagocytic activity of leucocytes. Vitamin B_1 has an action in macrophages and affects neutrophil motility. Vitamin B_6 can release cytokines or chemokines and has responsibility to the responsiveness of NK cells. In addition, vitamin B_{12} increases lymphocytes as well as improves the abnormal CD4/CD8 ratio and NK cell activity (Shetty *et al*, 2010; Spinas *et al*, 2015).

The use of probiotics and vitamin B_1, B_6, B_{12} supplementation in tuberculosis patient has not yet been determined. Therefore, this study was aimed to analyze the effects of those supplementation on IFN-γ and IL-12 levels in tuberculosis patients.

MATERIALS AND METHODS

The study protocol was ethically approved by the Ethics and Law Committee of Airlangga University Hospital on August 2016. The study was pre-post test randomised control by time series applied to the naïve and newly-diagnosed TB patients. The first group consisted of patients with the first-line standard therapy of oral antituberculosis and vitamin B_6 (control), whereas the second group was patients receiving therapy and supplementation of probiotics and vitamin B_1, B_6, B_{12} (intervention). Both standard therapy and supplementation were administered once daily during the intensive phase of treatment. The study was conducted at the Universitas Airlangga Hospital and Primary Healthcare Centers in Surabaya within period of December 2016 and February 2017. The inclusion criteria were patients aged 18-65 years, diagnosed with active TB, started first-line antituberculosis treatment for the intensive phase, had no previous history of probiotics and vitamin B_1, B_6, B_{12} before enrollment, and signed the informed consent. The exclusion criteria were patients with HIV co-infection, pregnancy, lactation, diabetes mellitus, pneumonia, and current administration of corticosteroids or other immunosuppressants.

Multistrain probiotics (*Lactobaccilus acidophilu*, *L. casei*, *L. rhamnosus*, *L. bulgaricus*, *Bifidobacterium breve*, *B. longum*, *Streptococcus thermophilus*), vitamin B_1 100mg, B_6 100mg, B_{12} 5.000mcg were administered once daily in the intervention (second) group. Blood samples were withdrawn at baseline, after one month, and two months of intensive therapy for further plasma IFN-γ and IL-12 assay using the ELISA method. The t-test analysis was performed to evaluate the effects of probiotics and vitamin B supplementation.

RESULTS AND DISCUSSION

The characteristics of patients (Table I) Data were distributed normally, and no significant differences were found between the two groups (p>0.05).

The Figure 1A and 2 A showed the plasma IFN-γ levels in both groups. The levels increased from 32.735pg/mL before therapy to 40.325pg/mL (*p*=0.445) after one month followed by a decrease to 39.640pg/mL (*p*=0.859) in the second month. Whereas, plasma IFN-γ levels in the intervention group rose from 73.767pg/mL before therapy to 97.768pg/mL (*p*=0.241) in the first month and next significantly declined to 43.472pg/mL (*p*=0.007) at the end of two-month of intensive therapy.

Similarly, figures 1B and 2B revealed that plasma IL-12 levels in the control group elevated from 2.663pg/mL before therapy to 3.303pg/mL (*p*=0.091) in the first month and diminished to 2.550pg/mL (*p* = 0.155) after two months of therapy. In addition, plasma IL-12 levels of the intervention group increased from 3.173pg/mL before therapy to 4.168pg/mL (*p*=0.061) after one and decreased significantly to 2.567pg/mL (*p*=0.003) in the second month.

Table I. Patients demography

Parameter	Control group N (%)	Intervention group N (%)	*p* value*
Total	11	11	
Gender			
Male	4 (36.4)	4 (36.4)	1.000
Female	7 (63.6)	7 (63.6)	
Age (years)			
18-24	2 (18.2)	2 (18.2)	
25-34	2 (18.2)	2 (18.2)	
35-44	2 (18.2)	5 (45.4)	0.389
45-54	4 (36.3)	1 (9.1)	
55-65	1 (9.1)	1 (9.1)	
AFB smear result			
Positive	7 (63.6)	6 (54.5)	1.000
Negative	4 (36.4)	5 (45.5)	
Duration of symptoms (cough, night sweats)			
≥ 1 month	9 (81.8)	11 (100.0)	0.065
< 1 month	2 (18.2)	0 (0)	
Smoking			
Yes	1 (9.1)	1 (9.1)	1.000
No	10 (90.9)	10 (90.9)	
Co-morbidities			
Asthma	0 (0)	1 (9.1)	
Hyperthyroid	0 (0)	1 (9.1)	0.413
Ulcer	1 (9.1)	2 (18.2)	
None	10 (90.9)	7 (63.6)	
Diagnosed TB			
Pulmonary	7 (63.6)	10 (90.9)	0.311
Extrapulmonary	4 (36.4)	1 (9.1)	

* calculated by chi-square test for categorical variables

This study recruited 22 newly-diagnosed TB patients and were divided equally into each group. The diagnosis was primarily based on the Acid-Fast Bacilli (AFB) smear results or chest x-ray, in addition to clinical features such as cough and night sweats. Although most patients experienced pulmonary TB rather than extrapulmonary type, the process of body immune system response to Mtb for both conditions is similar (Adrian *et al*, 2015). All patients were administered the first-line antituberculosis therapy as standard therapy according to the current WHO guideline (WHO, 2017).

Probiotics are microorganisms alive that given in sufficient quantities to provide benefits. They have shown to increase the amount and activity of cytotoxic NK cells in peripheral blood, with IL-12 immunomodulation increasing the expression of NK cell receptors. Thus, it can be concluded that probiotics are capable of increasing Th1 responses (Kechagia, *et al*, 2013; Nanno, *et al*, 2014). For example, the *L. pentosus* strain significantly increased the activity of NK cells in the spleen through IL-12. Increased levels of IL-12 in dendritic cells cause NK stimulation, thus increasing IFN-production (Forsythe, 2013; Mortaz, *et al*, 2013). Previous studies have shown that combination of multistrain probiotics provide better results because they produce synergistic effects among strains to inhibit more pathogens. Another study also revealed that the use of probiotics is useful in reducing the incidence of respiratory tract infections (Chapman *et al*, 2011; Chapman *et al*, 2012).
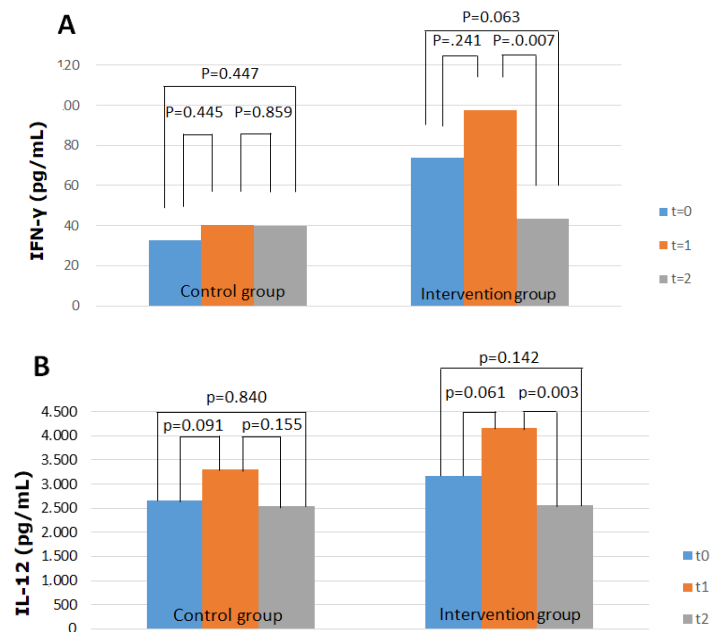
Figure 1. Changes in plasma IFN-γ (A) and IL-12 (B) levels in the control and intervention groups before therapy (t0), after one month (t1), and two months of intensive phase therapy (t2).
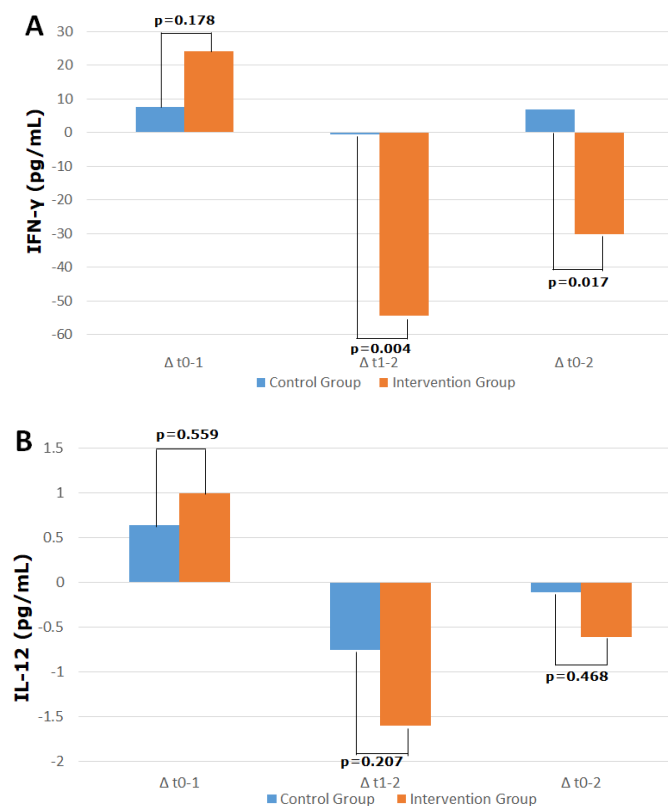

Figure 2. Differences within a month between IFN- γ and IL-12 levels in in the control and intervention groups during the intensive phase therapy

Vitamins also affect the body's immunity, such as vitamin B_1 which works on macrophages and influences the neutrophil motility, vitamin B_6 is associated with the release of cytokines or chemokine's and responsiveness of NK cells, and vitamin B_{12} influences lymphocytes, T cell proliferation, CD4/CD8 and NK cells activity (Shetty *et al*, 2010; Spinas *et al*, 2015).

Furthermore, combination of multistrain probiotics and vitamin B supplementation provides enhanced immunomodulation activities during the intensive phase therapy as shown by the trends of increase in the first month and decrease at the end of intensive therapy between IFN-γ and IL-12 levels in the intervention group compared to the control group. Clifford *et al* (2015) reported that there was an increase in IFN-γ levels in active TB treatment. When the host is infected by Mtb, there will be bacterial influx by phagocytic cells, especially alveolar macrophages and neutrophil, which in turn induce apoptosis. The apototic cells will be captured by pulmonary dendritic cells that act as antigens presenting cell (APC) to be carried into lymph with the help of IL-12 and chemokines. Dendritic cells also produce IL-12 to support differentiation of naive T cells into helper T cells (Th1, Th2, TTh9, Th 17, Th 22 and T regulator cells). In the lung, T cells produce various cytokines such as TNF-α and IFN-γ by Th1 cells (O'Garra *et al*, 2013). In early infection phase and first month of antituberculosis therapy, probiotics and vitamin B supplementation will enhance IFN-γ production, but further the IFN-γ level will gradually decrease along with the decrease of inflammation process at the end of intensive treatment (Abul *et al*, 2016).

Indeed, the levels of IFN-γ were higher in the first month of the intervention group compared to the control. This suggested that the supplementation of probiotics and vitamin B_1, B_6, B_{12} plays a role in the modulation of IFN-γ secretion in response to Mtb infection. Increased IFN-γ levels in the first month may enhance bacteria eradication, as the result the bacteria in the lung or extrapulmonary sites will decrease resulting reduced systemic inflammation. Therefore, at the end of intensive therapy the levels of IFN-γ were

diminished as shown by a significant reduction in the intervention group. Nevertheless, Devici *et al* (2005) stated that there was a significant increase in IFN-γ levels in the second month and then decreased in subsequent months.

Interestingly, the similar trends also applied to the IL-12 levels. They increased in the first month and then decreased in the second month of therapy either in control or intervention groups. However, the net differences showed greater in the intervention group rather than in the control. IL-12 supports Th1 cell defferention whose secretes IFN-γ, and then IFN-γ will stimulate NK cell to produce more IFN-γ (Forsythe, 2011). Sai Priya *et al* (2009) revealed a decrease in IL-12 response during treatment. This suggested that supplementation may help increase levels of IL-12 in the first month, regulate the balance of T-cell responses and stimulate NK cells in the spleen through IL-12 and increase production IFN-γ (Spinas *et al*, 2015). IFN-γ will in turn help to eradicate the bacteria. Reduced levels of IL-12 were aligned with the levels of IFN-γ. At the end of intensive therapy, the number of active Mtb has decreased, thus reducing the levels of IFN-γ and IL-12 levels.

Last, this study was conducted in limited number of patients, so the results cannot be directly generalized to greater population. Therefore, multi-center study with more subject is needed.

CONCLUSIONS

Supplementation of once-daily multistrain probiotics and vitamin B_1, B_6 and B_{12} could improve immune system through IFN-γ and IL-12 modulation, increase plasma levels of IFN-γ and IL-12 in early phase to help speed up Mtb eradication during the intensive phase of tuberculosis treatment.

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