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Effect of Combined Probiotics and Zinc Supplementation on Immune Status of Pulmonary Tuberculosis Patients

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Abstract: Patients with pulmonary tuberculosis generally malnourished due to side effect in anti-tuberculosis drugs, which altering gastrointestinal tract, it affects on recovery process, immune system and response. Probiotics and zinc are thought to have beneficial effects for nutritional status and immune response. This study was conducted to analyze the effect of combined probiotics and zinc on levels of lymphocytes, neutrophils lymphocyte count ratio (NLR) and monocytes in patients with pulmonary tuberculosis in Lung Health Center of Semarang. In a quasi-experimental study (quasi experiment) randomized design with pre-post test control group design, fifty four pulmonary tuberculosis patients were divided into 2 groups, the treatment group received a combination of probiotics and zinc, as well as a control group given a placebo, administration for 4 weeks. There were no differences in mean age, levels of lymphocytes and monocytes NLR at baseline ($p>0.05$). Increased levels of lymphocytes and decreased levels of monocytes and NLR after administration of the combination of probiotics and zinc ($p<0.05$). There was no significant relationship between nutriens intake with high levels of lymphocytes, NLR and monocytes ($p>0.05$). The most powerful effectiveness of the combination probiotics and zinc contained in the lymphocyte levels (12.8%). It is concluded that the combined probiotics and zinc over 4 weeks improve the immune system of patients with pulmonary tuberculosis measured by lymphocytes, NLR and monocytes.

Key words: Combined probiotics and zinc, lymphocytes, nlr, monocytes, pulmonary tuberculosis

INTRODUCTION

Background: Indonesia currently has the third highest number of pulmonary tuberculosis (TB) cases in the world, after India and China, with an average of 325.582 cases in 2013 (WHO, 2003). A report by Riskesdas in 2013 indicated the prevalence of TB patients in Central Java was ranked fifth, with pulmonary TB cases being highest in Lung Health Center of Semarang (Profil Kesehatan Kota Semarang, 2014).

TB patients generally experience a decrease in nutritional status, resulting from the adverse effects of anti-TB drugs that cause nausea, vomiting, diarrhea and loss of appetite. These drugs are also allegedly detrimental to microflora in the digestive tract, weakening the immune system. One study reported that most pulmonary TB patients experienced chronic protein/energy, zinc and retinol plasma deficiency (Pakasi *et al*., 2010). Zinc functions as an antioxidant which neutralizes free radicals by means of electron donors. Several studies have shown that zinc supplementation in patients with pulmonary TB can improve their nutritional status, hemoglobin levels, zinc plasma, retinol plasma, body fat, as well as strengthen the immune system of patients with human immunodeficiency virus (Pakasi *et al*., 2010; Rahfiludin and Pradigo, 2012; Dharmana *et al*., 2007).

Monocytes, neutrophils and lymphocytes are cells that play an important role in the defense of specific and nonspecific. Antigen-presenting cells produce cytokines and attract and activate lymphocytes and macrophages to destroy microbes and infected cells (Abbas *et al*., 2007; Baratawidjaja and Rengganis, 2009). Individually, probiotics and zinc have been shown to have beneficial effects on patient nutritional and immune status; combined, their positive effects have been shown to be stronger, increasing mineral absorption and enhancing the immune system (Surono *et al*., 2014). Herein, we investigated whether supplementation with a combination of probiotics and zinc effects lymphocyte levels, neutrophil: lymphocyte ratios (NLR) and monocyte levels in patients with pulmonary TB at Lung Health Center of Semarang.

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Objective: To analyze the effect of combined probiotics and zinc supplementation on lymphocyte levels, NLR and monocyte levels in patients with pulmonary TB at Lung Health Center of Semarang.

METERIALS AND METHODS

Our quasi-experimental study (quasi experiment) had a randomized design with pre-post test control groups. Respondents included pulmonary TB patients on anti-TBdrug therapies that met inclusion and exclusion criteria. A total of 54 respondentswere randomly divided into two groups and given one capsule containing either probiotics + zinc (TB treatment group) or placebo (TB control group) a day for 4 weeks. Measurement of lymphocyte levels, NLR and monocyte levels before and after supplementation was completed using aflow cytometric method with a hematology analyzer tool. Nutrient intake made a mean of three-day food recalls method and calculated using the Nutrisurvey program (2007) and compared with Indonesian Dietary Allowances 2013. Primary and secondary data from respondents were obtained from interviews, clinical/laboratory measurements, etc.collected at Lung Health Center of Semarang.

Univariate analysis was used to determine the distribution of each study variable, while normality testing was completed using the Kolmogorov-Smirnov test. Bivariate analysis was used to determine the effect of probiotics + zinc on lymphocyte levels, NLR, monocyte levels and nutrient intake. Normally distributed variables were analyzed by independent sample t-test, while data with a nonnormal distribution were analyzed by Mann-Whitney test.

RESULTS

The majority of respondents in both groups were males and had junior high school education background. Most of respondent in the control group had were entrepeneurs (55.6%), while those in the treatment group were private employees (55.6) (Table 1).

Statistical analysis showed no differences in mean age, lymphocyte levels, NLR, or monocyte levels at baseline ($p>0.05$) (Table 2).

The mean intake of energy, protein and zinc in the TB control group was higher than the treatment group. There was no difference in nutrient intake between the groups, with the exception of vitamin A intake ($p = 0.045$). The energy, protein and vitamin A consumption ratesin TB controls was higher than in the treatment group, while the rate of zinc intake was lower. The average levels of energy, protein,and zinc consumption in both groups were considered deficient (<70%), while the level of vitamin A consumption in TB controlswas in the middle (80-90%) but deficient in the treatment group. Furthermore, nutrient consumption levels were not significantly different between the groups ($p>0.05$) (Table 3).

Statistical analysis showed significantly different lymphocyte levels between the groups ($p = 0.013$), as well as significant differences in lymphocyte levels before and after supplementation ($p = 0.002$). NLR levels were not significantly different between groups, but there was a significant difference before and after supplementation ($p = 0.008$). The mean NLR at the beginning and end of the study decreased in both groups. Monocyte levels were different between the groups ($p = 0.04$) and there was a significant difference in monocyte levels before and after the 4-week supplementation with probiotics + zinc ($p = 0.02$). The mean level of monocytes at the beginning and end of the study decreased in both groups (Table 4).

The relationship between nutrient intake and high lymphocyte levels, NLR differences and monocyte levels was not significant ($p>0.05$) (Table 5).

After supplementation for 4 weeks, there was no significant difference in the outcome between the two

Table 1: Characteristics of respondents

	Treatment		Controls	
Characteristics of respondents	Amount	Percent (%)	Amount	Percent (%)
Gender				
Man	21	77.8	15	55.6
Women	6	22.2	12	44.4
Last education				
No School	9	33.3	3	11.1
Elementary	3	11.1	1	3.7
Junior High School	12	44.4	15	55.6
Senior High School	-	-	2	7.4
College	3	11.1	6	22.2
Work				
Not working	-	-	2	7.4
Housewife	2	7.4	2	7.4
Labour	3	11.1	-	-
Prifate employee	5	18.5	15	55.6
Self employee	15	55.6	7	25.9
Civil servant	2	7.4	1	3.7

Table 2: Respondent characteristics in early research

	Treatment				Control				
Characteristics of respondents	Med	Min	Max	Mean±SD	Med	Min	Max	Mean±SD	p
Age (y)	44	18	60	43.22±14.23	43	18	60	41.56±11.70	0.579[b]
Lymphocyte (µL)	1.90	0.60	4.40	2.00±0.92	2.00	0.50	3.20	1.95±0.57	0.819[a]
Neutrophil lymphocyte count ratio (NLR) level (µL)	3.00	1.08	8.60	3.93±2.39	3.00	1.31	22.20	4.14±3.95	0.924[b]
Monocyte level (µL)	0.80	0.30	2.20	0.96±0.45	0.90	0.30	1.40	0.83±0.33	0.602[b]

[a]Independent [b]Mann-Whitney

Table 3: Average nutrient intake substances and consumption

	Treatment				Control				
Variable	Med	Min	Max	Mean±SD	Med	Min	Max	Mean±SD	p
Intake									
Energy (kkal)	1220.83	548.40	1566.86	1178.98±261.76	1294.70	503.70	1783	1236.70±358.81	0.50[a]
Protein (g)	35.70	14.26	52.86	35.60±8.94	35.70	17.30	67.50	38.13±12.55	0.39[a]
Vitamin A (µg)	223.50	8.70	1460.25	329.07±304.96	396	72.43	1358.70	228.98±191.66	0.04[b]
Zinc (mg)	4.75	2.20	6.96	4.41±1.125	4.50	2	7.3	4.42±1.23	0.99[a]
Consumption (%)									
Energy	50.60	20.50	63.45	49.11±10.63	54.96	21.66	78.65	52.11±14.75	0.39[a]
Protein	59.27	21.93	81.32	57.17±14.11	54.90	26.61	120.53	60.07±21.25	0.55[a]
Vitamin A	39.28	1.45	292	57.88±57.56	66	12.07	226.45	89.84±67.12	0.50[b]
Zinc	38.46	12.94	53.53	35.91±9.48	35.76	15.38	73	35.46±11.82	0.87[a]

[a]Independent [b]Mann-Whitney

groups ($p>0.01$). The most powerful and effective combination of probiotics and zinc is lymphocytes 12.8% (Table 6).

DISCUSSION

TB in Indonesia is commonly found in men because of their increased tendency to consume cigarettes and alcohol; cigarette consumption can damage lung defenses restricting their ability to fight germs that have entered the airways. Moreover, those who have jobs outside the home are at a greater risk of coming in contact with people with TB (Hickson *et al.*, 2007). World Health Organization data suggests that cases of pulmonary TB in developing countries are abundant between the ages of 15-29 years (WHO, 2003). Research by Panjaitan (2014) indicated the age range for that pulmonary TB patients in general is from 18 to 59 years (77.8%). These productive ages are the result of high mobility, which puts them at greater risk for contracting TB outside the home.

The increase in vitamin A intake in the TB treatment group was likely due to zinc supplementation as this metal plays a role in the regulation of vitamin A metabolism. In particular, zinc controls the intercellular and intracellular transport of retinol via retinol binding protein and also acts as a cofactor in the synthesis of an enzyme that regulates absorption and function of vitamin A (Scrimshaw *et al.*, 1968). Karyadi *et al.* (2000) showed that malnourished individuals had a 3.7-fold increased risk of suffering from pulmonary TB than those with sufficient nutrition. Nutrition affects the body's immune defenses by increasing its resistance to disease. Malnutrition, as well as lack of energy,protein, vitamins, iron and other substances also affect the body's immune resistance, causing it to be more susceptible to various diseases such as TB. Malnutrition can result from an imbalance in the quality and quantity nutritional intake and can also result from chronic infectious diseases (Karyadi *et al.*, 2000). For example, TB can lead to malnutrition due to a changes in the metabolic process and side effects of anti-TB drugs (nausea, vomiting, diarrhea and loss of appetite). One study reported that most pulmonary TB patients experience chronic protein/energy deficiency, zinc deficiency and retinol plasma (Pakasi *et al.*, 2010).

Lymphocytosis and/or increased lymphocyte levels represent normal immune responses against TB. This response raises lymphadenopathy and an increase in circulating lymphocytes; below normal lymphocyte levels suggests the body is fighting infection. Active TB causes a decrease in the total number of T and B cells due to a decrease in T4 cells (Patiung *et al.*, 2014). Research by Wahyuningsih (2012) showed increased lymphocyte levels after supplementation with the probiotic Lacidofil™ for 4 weeks. Suparman *et al.* (2011) suggested that supplementation with milk, synbiotic and micronutrients can improve the immune system by increasing serum

Table 4: Effect combination supplementation (Probiotics and Zinc) against kadar lymphocytes, lymphocyte neutrophil count ratio (NLR) and monocytes

	Treatment			Control			
Variable	Min	Max	Mean±SD	Min	Max	Mean±SD	p
Lymphocyte							
Before treatment	0.60	4.40	2.00±0.92	0.50	3.20	1.95±0.57	0.819[a]
After 4 weeks	1.20	5.00	2.21±0.86	0.70	3.60	1.89±0.60	0.123[a]
ΔLymphocyte	-0.70	0.90	0.21±0.35	-1.20	1.10	-0.06±0.41	0.013[a]
p			0.002[c]			0.438[c]	
NLR							
Before treatment	1.08	8.60	3.93±2.39	1.31	22.20	4.14±3.95	0.924[b]
After 4 weeks	1.17	15.50	3.72±3.03	1.10	10.85	3.41±2.22	0.938[b]
ΔNLR	-3.99	10.36	-0.2±2.40	-11.35	0.90	-0.72±2.26	0.239[b]
p			0.008[c]			0.097[c]	
Monocyte							
Before treatment	0.30	2.20	0.96±0.45	0.30	1.40	0.83±0.33	0.602[b]
After 4 weeks	0.30	1.80	0.83±0.40	0.20	1.50	0.80±0.34	0.899[b]
ΔMonocyte	-1.10	1.00	-0.12±0.37	-0.40	0.30	-0.03±0.03	0.040[b]
p			0.026[c]			0.303[c]	

[a]Independent, [b]Mann-whitney, [c]Wilcoxon

Table 5: Relationship Intake Levels Eating with lymphocytes, Neutrophile Lymphocyte Count Ratio (NLR) and monocytes

	Treatment			Control		
	p-value					
Variable	Lymphocyte	NLR	Monocyte	Lymphocyte	NLR	Monocyte
Energy (kcal)	0.875	0.471	0.324	0.634	0.911	0.267
Protein (g)	0.809	0.457	0.386	0.676	0.719	0.766
Vitamin A (µg)	0.781	0.999	0.376	0.169	0.252	0.207
Zinc (mg)	0.775	0.491	0.505	0.848	0.804	0.611

[a]Pearson

Table 6: Differences changes against intervention levels lymphocytes, lymphocyte neutrophil count ratio (NLR) and monocytes

	Group				
Variable	Treatment	Control	F	p[£]	Partial eta squared
Lymphocyte	2.21±0.86	1.89±0.61	7.51	0.01	0.128
Neutrophil limphocyte count ratio (NLR)	3.73±3.03	3.41±2.22	0.72	0.40	0.014
Monocyte	0.84±0.40	0.80±0.37	0.55	0.46	0.011

£General Linear Model is controlled by the results of the initial inspection of each variable

vitamin A levels. Neutrophils are the first cells to target bacteria that enter the body and most circulating leukocytes are neutrophils. NLR levels are thought to be a stronger predictor of bacteremia compared with discrimination-based neutrophilia or lymphocytophenia only. Research conducted by Neul *et al.* (2012) indicates that an increase in total white blood cells and neutrophils indicates the occurrence of inflammatory reactions due to bacterial infection. Moreover, Basem *et al.* (2012) suggests NLR levels are associated with a higher risk of death in patients with breast cancer.

Cells that are mainly involved in nonspecific immune defense include mononuclear cells (monocytes and macrophages) and polymorphonuclear cells or granulocytes. Monocytes act as antigen-presenting cells, recognizing and attacking microbes and cancer cells and also produce cytokinesin response to infection. Polymorphonuclear cells or granulocytes represent 60-70% of all normal white blood cells and include neutrophils, eosinophils and basophils. These cells play a role in acute inflammation (Baratawidjaja *et al.*, 2009).

The mechanism by which probiotics inhibit growth of pathogenic bacteria in the intestinal mucosa may involve their competitive attachment to enterocytes. Enterocytes that have been saturated with probiotic bacteria are unable to attach topathogenic bacteria. Thus, the presence of probiotic bacteria in the intestinal mucosa may prevent colonization of pathogenic bacteria.In addition, lactic acid bacteria attached to intestinal epithelial cells can activate macrophages, stimulate production of interlukins and increase the cell proliferation activity of lymphocytes (Firmansyah, 2001; Ouwehand *et al.*, 1999). Probiotics can stimulate cytokines and other mediators as result of an increase in cell-mediated effector function, such as increased phagocyte function and production of IFN-γ. Another benefit of probiotics is that they help reduce inflammatory responses seen in Crohn’s disease and with food allergies by increasing anti-inflammatory cytokine production and reducingproduction of proinflammatory cytokines, there by strengthening the intestinal mucosal barrier (Lactobacillus Rosell-52, 2015).

Research conducted by Sari *et al.* (2014); Rahfiludin *et al.* (2012) and Abbas *et al.* (2007) demonstrates that zinc supplementation in patients with pulmonary TB can improve their nutritional status, hemoglobin levels, zinc plasma, retinol plasma, body fat and improve immunity in patients with human immunodeficiency virus as evidenced by increased levels of CD4+. Zinc supplementation can increase the production of cytokines by helper T cells, promoting cellular proliferation and differentiation. Cytokines play many roles in the immune response,including activation of T cells, B cells, monocytes and macrophages (Prasad *et al.*, 2007).

Research by Hatta (2011) distinguished the effect of combination probiotic and zinc supplementation versus supplementation with zinc alone and showed that the combination of probiotics and zinc more effectively reduced the severity of acute diarrhea in children under five Surono *et al.* (2014) reported that preschool children provided a combination of probiotics and zinc for 90 d had an improved humoral immune system as evidenced by a significant increase in serum zinc levels. These result suggest the combination of probiotics and zinc has a synergistic effect, increasing digestion and absorption of nutrients, including the absorption of zinc. However, Wigiyandiaz (2013) showed no significant difference between the intake of vitamins A, C and E, selenium and zinc in elderly persons with infectious diseases. The relationship was not significant between nutrient intake and variations in research outcomes may result from the duration of supplementation with probiotics and zinc. Furthermore, supplementation with other necessary nutrients may also affect lymphocyte levels, NLR and monocyte levels.

Conclusion: The combination of probiotics and zinc for 4 weeks can improve the immune system of patients with pulmonary TB as indicated by increased lymphocyte levels, NLR and monocyte levels.

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