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The Effect of Zinc, Lysine, and Vitamin A Supplementation to Increase Cellular Immune Response of Pulmonary Tuberculosis Patients

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Abstract

Background: Tuberculosis stills off be found coincides by malnutrition's condition, which is deficiency among macro and micro nutrient. In the normal circumstances nutrient can be sufficed from knock about food, but in poverty condition and chronic disease, not all nutrient component can be accomplished. So that it's required to the nutrient supplements substance in order to that nutrient requirement can be accomplished, either in macronutrient or micronutrient.

Objective: We studied the immune cellullar's response (total of TCD4 and the level of INF-γ) on pulmonary tuberculosis patient through supplements zinc, lysine and vitamin A.

Methods: In a double-blind randomized community trial, new sputum smear positive pulmonary tuberculosis patients were assigned randomly to zinc+lysine, zinc+lysine+vitamin A, and placebo. Patients examined albumin, retinol and saliva zinc level, CD4 and IFN-γ level before treatment and after 2 months of treatment.

Results: The result of the observation shows that giving zinc supplement, lysine, and vitamin A. which is done every day on pulmonary tuberculosis patient up to 2 months can increase the amount of TCD4 as meant (p = 0,040; = 0,050), and IFN- γ rate is increases as meant (p = 0,036; = 0,050).

Conclusion: This study has found that supplementation of zinc, lysine, and vitamin A for 2 months can increase total TCD4 and the levels of IFN- γ in pulmonary tuberculosis patients.

Keywords: Zinc; Lysine; Vitamin A; Immune response; Pulmonary tuberculosis

Background

Indonesia constitutes one of 22 states that still stayed at fifth thread after India, china, South of Africa and Nigeria in term of TB's patient amount outgrown at the world (High Burden Countries) [1].

Tuberculosis often coincides by malnutrition's condition, which is deficiency among macro and micro nutrient (such is use: protein, zinc, vitamin, iron substance A and vitamin C). Of 87% TB's mature Patients, they experience malnutrition with their body weight vicinity 30-50 kg with IMT approximately 16,4, album serum 3,8 g/dl, hemoglobin 11,00 g/dl, plasma zinc 11,8 mol/l and retinol is plasmas 0,7 umol/l [2]. One of factor what do may be can cause someone can become patient of pulmonary tuberculosis is low body resistance, one that because of bad nutrient. There is a big circle which has an interaction between less nutrient with pulmonary's tuberculosis disease, which is person with less nutrient state will be easily most tuberculosis lung's infection.

On TB's infection occurs the respond immunology as cellular's immunity. Cellular's immunity causes proliferation lymphocytes TCD4 and producing local sitokin. As response to antigen that issued by M. tuberculosis lymphocytes TCD4 influences lymphocytes T Th1 to activate macrophage. This Sitokin will pull monosit blood goes to TB's lesion and activates it. The active Monosit or macrophage and lymphocytes $TCD4^+$ produce lisosom enzyme, radical oxygen, nitrogen intermediate notably nitrogen oxide and Interleukin-2. This oxide nitrogen is being activated by TNF α and INF γ to constrain growth and killing M. tuberculosis one that virulent.

The sufficiency of Diet is not only enhances patient nutrient state, but also has an influential on immunity system that helps in healing TB. In the normal circumstances, the nutrient can be sufficed from knockabout food but in condition poverty and chronic disease, are not all nutrient component can be accomplished. Supplementation of zinc, lysine, and vitamin A are expected can synergy with a good consideration, and can be used by TB's patient to help them during velocity cure time, the enhancement of immune's system, and nutrient state of tuberculosis's patient.

Methods

Study design, time and location

The design of the study was a randomized controlled trial pre and post's test control's test design's group, with supplied double blind as a treatment, in which a treatment code was given to a subject. Every district had its random allocation.

Patients were given standard treatment for tuberculosis and randomly divided into three supplementation groups: zinc + lysine, zinc + lysine + vitamin A, and placebo. The supplementation was taken daily and patients were followed up until 2 months.

The primary outcome of the study was total of TCD4 and the level of INF-γ. Secondary outcome were: nutritional status (BMI) results of blood examinations (albumin and vitamin A) and saliva examinations (zinc level).

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The study was conducted at pulmonary's Hospital (BP4) of Surabaya East Java Province, Indonesia, from September 2011 until February 2012.

Subjects and sample size

Subjects were newly diagnosed sputum smear-positive (SS+) TB patients aged 15-54 years. Prior the study conducted screening: pregnant or lactating females, smoker, who had underlying chronic or degenerative disease as HIV and Diabetes mellitus, and suffer intestinal worm were excluded. All eligible patients were given information regarding the study including problem that might occur; and were asked to sign an informed consent for their participations in study. The sample size was calculated based on the ability to determine a difference with = 0.05.

Micronutrient supplementation and anti-TB drugs

Supplements and placebo were prepared by pharmaceutical laboratories pharmacy faculty Surabaya Airlangga University, Indonesia, in the form of capsules. Each micronutrient capsule contained 15 mg zinc (as zinc sulfate), lysine 50 mg and/or 1500 retinol equivalents (5000 IU) in a lactose matrix. Dosage of zinc was determined based on the recommended daily allowances for adult Indonesia. The placebo capsule consisted of lactose alone. All capsules were similar in term of shape, color and size. Standard TB drugs were based on WHO guide lines, comprising 300 mg isoniazid, 450 mg rifampicin, 1500 mg pyrazinamide and 750 mg ethambutol daily for 2 months.

Data collection

All patients underwent physical examination nutritional and food intake assessment as described in other study, saliva, and blood analyses before the treatment started, and at the end of the treatment.

Nutritional status

Nutritional status was determined based on anthropometric measurements and micronutrient concentrations in the plasma, serum, and saliva. The anthropometrics measurements were: body weight, height, body mass index. Body weight was assessed using an electronic platform model weighing scale (770 alpha; SECA, Hamburg, Germany) to the nearest 0.1 kg. Height was recorded to the nearest 0.1 cm using a microtoise. BMI was calculated as body weight divided by height squared (kg/m^2).

Saliva examination

Saliva samples taken in the morning as much as 5 mL. Examination using analyzed atomic absorption spectrometry (ASS) with normal value 88-135 mg/L.

Blood examination

Blood samples were collected between 08.00-11.00 AM in the local health center. Approximately 12 mL of whole blood was withdrawn and separated into vacutainers containing EDTA and heparin. Vitamin A level dan IFN- level were measured in serum using enzyme-linked immunosorbent assay (ELISA) method, and total TCD4 was measured in plasma. Plasma was separated after centrifugation at 750 x g for 10 minutes at room temperature using cytometer. Serum albumin was measured using Spectrophotometer (Microlab 300, Merck, Germany) with a normal range 35-52 g/L.

Statistical analysis

A one-sample Kolmogorov-Smirnov test was used to determine whether the variables were normally distributed. Data on the characteristics of subject at enrollment for their age and gender distribution, nutritional status, and results blood concentrations were summarized and used to assess the comparability of the patients randomly assigned to the three treatment groups. Different means between groups were tested for significance using one-way ANOVA when normally distributed, and Kruskal-Wallis test when not normally distributed. Within group changes were tested using paired student-t test for normally distributed data and the Wilcoxon signed-rank when than 0.05 were significant. An intention to treat analysis was applied. Statistical analyses were performed using computer software for Window PC version 16.0.

Ethical consideration

The research proposal was approved by Ethics Committee of the Faculty of Medicine, Surabaya Airlangga University, Indonesia. Ethical clearance number: 029/EC/KEPK/FKUA/2011. Data were collected after subjects agreed to participate in the study and gave written informed consent voluntarily.

Results

Variables Groups	Groups		
	1 (n = 10) (Zinc + Lysine)	2 (n = 10) (Zinc + Lysine + V.A)	3 (n = 10) (Placebo)
Age (year), mean ± SD	31 (± 13.08)	34 (± 15.07)	35 (± 13.07)
Gender			
Male	8	7	7
Female	2	3	3
Education			
Primary	2	3	3
Secondary	5	3	4
Tertiary	3	4	3
Occupation			
Private	9	9	10
Teacher	_	1	_
PNS	1	_	_
Income per month			
<1 million	5	6	5
1 – 2 million	3	2	4
>2 million	2	2	1
Weight (kg)	43.53 (± 5.88)	43.13 (± 4.80)	43.51 (± 8.13)
BMI (kg/m2)	17.05 (± 2.82)	17.77 (± 1.12)	17.74 (± 4.13)
Albumin (g/L)	3.87 (± 0.52)	3.84 (± 0.35)	3.84 (± 0.64)
Saliva zinc level	43.77 (± 14.03)	45.28 (± 9.03)	49.53 (± 8.99)
Retinol level	6.31 (± 3.23)	7.77 (± 4.38)	6.09 (± 3.19)
Energy consumption	1259.2 (± 172.5)	1439.2 (± 190.4)	1439.2 (± 190.4)
	70.09% RDA	74.27% RDA	78.18% RDA
Protein consumption	46.91 (± 8.12)	50.51 (± 8.07)	50.51 (± 8.07)
	68.71% RDA	69.51% RDA	73.2% RDA
Fat consumption	44.59 (± 6.83)	46.45 (± 3.08)	46.45 (± 3.08)
	86.53% RDA	86.3% RDA	90.8% RDA
Vitamin A consumption	496.70 (± 35.15)	483.40 (± 39.18)	483.40 (± 39.18)
	85.64% RDA	86.32% RDA	86.3% RDA
Zinc consumption	8.86 (± 2.69)	8.52 (± 1.52)	8.29 (± 1.25)
	70.6% RDA	70.8% RDA	70.8% RDA 68.71% RDA
CD4	703.5 (± 190.3)	597.2 (± 139.3)	568.2 (± 244.6)
IFN-gamma (pg/ml)	4.54 (± 0.94)	4.44 (± 0.64)	4.66 (± 0.98)

a = One-Way Anova; b = Average (± standard deviation)

Table 1: Base-line characteristics of study subjects (Data is shown as mean ± SD).

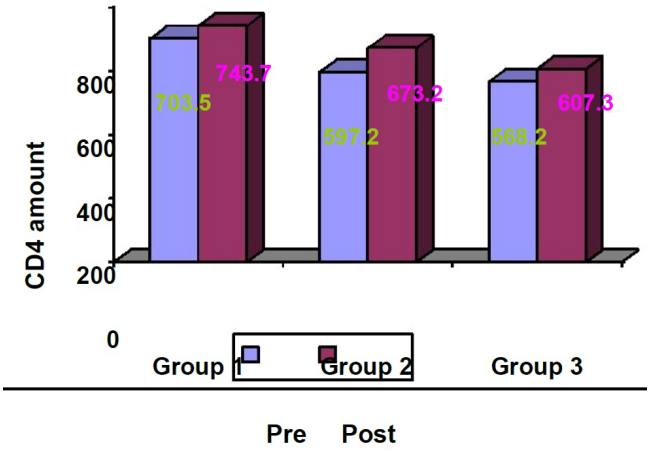

Figure 1: The differences of CD4'S total average in the first and the end of research at Pulmonary Hospital (BP4) Surabaya, in 2012.

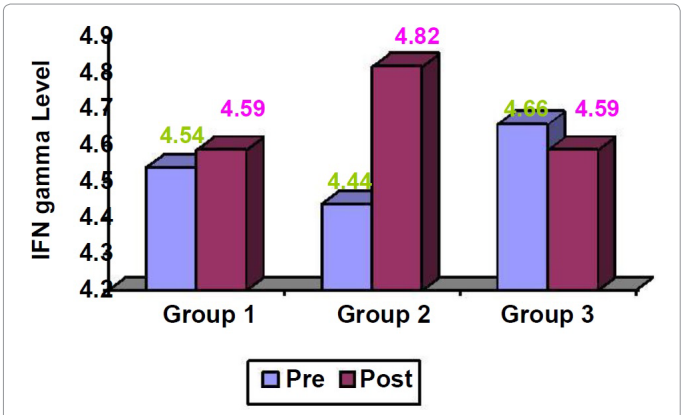

Figure 2: The differences of IFN gamma-content average in the first and the end of research at Pulmonary's Hospital (BP4) Surabaya, in 2012.

Discussion

IFN-γ plays very important role and central in immunity to Mycobacterium tuberculosis. IFN-γ produced by T-cell and NK cell and APC cell (in this case here are macrophage cell and the IFN-γ dindrit). IFN-γ is the main macrophage's activator. The Important features of IFN-γ are: 1). Potential activator from fagosit cells mononuclear, as MAF (macrophages activating factors), 2). Increasing MHC molecule expression in class I and II, 3). Cause directly cell differentiation of T and B cell, 4). Activating NK cell, neutrophil and endothelial vascular cell so that the adesi energy to T CD4+ cell increasing.

The result of research shows that giving by zinc, lysine supplement and vitamin A on lung's tuberculosis patient (group 2) for 2 months are able to increase IFN gamma-content as meant ($p = 0.036$; $<\alpha = 0.05$) among before and after conducting with the difference 0.38 pg /ml (± 0,49). Difference IFN gamma-content are more than the group which given by zinc, lysine supplement and also placebo's group.

Zinc has important role performing logistic of cell mediated immunity (CMI), especially, in thyme dependent lymphocytes (T cells). The use of zinc is important for proliferation and invulnerability cell differentiation, and for lymphocyte function engages in resistance mycobacterium tuberculosis germ, including production of IgG, interferon (INF)-gamma and tumor necrosis factor (TNF)-alpha, and microbicidal macrophage activity. Zinc deficiency will constrain NK cell function even for target cell desolation or in producing INF-γ that will affect macrophage activity.

IFN-γ that produced at the various cells of immune's system is the main sitokin of MA IFN-γ C and play roles especially in non-specific's immunity and cellular's specific. IFN-γ is sitokin that activates macrophage to kill germ. IFN-γ stimulating expression of MHC-1 and MHC II and APC Co stimulator. IFN-γ increasing CD4 IFN-γ cell differentiation naive to subset Th1's cell and prevents proliferation Th2's cell. IFN-γ work to cell B in sub-class IgG's shift Fcy's obligatory R on fagosit and activates the complement. Both of the process increases phagocytosis microbe that opsonisasioned. IFN-γ can shift Ig that participates in microbe elimination. IFN-γ activating neutrophil and stimulates sitolitik's effect NK's cell. IFN-γ activating fagosit and APC and shift induction B cell (isotip is antibody who can tie-up complement and Fc-R on fagosit, that's contrast with isotip that inducted by IL-4, indirect Induction of effect Yr 1 as a role of IL 12 and express receptor.

Zinc Supplement causes decreasing Immunopathology and helps conventional cure as more effective. Lysine increases to respond immune which is marked on thymus's addition size, and increasing T cell.

Some research point out that vitamin A's role in regulation secrecy IL 10. IL 10 which resulted by Th2 cell Helper constrains synthesis from sitokin Th1 Pro inflammation types, including IFN-γ and IL 2, even though in T cell and NK. giving vitamin A and zinc on TB's patient full age after 2 and 6 months will impacted on increasing body weight, IMT, LILA, triceps' fat thick and biceps, increasing fat proportion of the body, albumin-content, hemoglobin, decreasing of c reactive protein content and increasing plasma zinc.

Giving Zinc, lysine, and vitamin A on this research points out the result that's enhancement in interferon gamma's level (IFN-γ) on pulmonary's tuberculosis patient after supplementation for 2 months.

Conclusion and Suggestion

The research result: 1). There is total enhancement of TCD4'S cells in a mean manner ($p = 0.040$; = 0,050), on pulmonary tuberculosis patient after supplementation zinc, lysine, and A's vitamin; 2). There is totals enhancement of IFN'S in a mean manner ($p = 0.036$; = 0,050) on pulmonary tuberculosis patient after supplementation zinc, lysine, and vitamin A.

Suggestion: This research result ought to gets to be utilized for patient cure menagerie lung's tuberculosis, Besides given Anti Tuberculosis drug, it also notices in giving additional zinc, lysine, and vitamin A.

References

1. WHO (2010) Global Tuberculosis Control: WHO report.
2. Pakasi TA, Karyadi E, Suratih NM, Salean M, Darmawidjaja N, et al. (2010) Zinc and Vitamin A supplementation fails to reduce sputum conversion time in severely malnourished pulmonary tuberculosis patients in Indonesia. Nutr J 9: 41.

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