

The Effect of Zinc Sulphate on the Increase of CD4 T Lymphocytes in HIV/AIDS Patients

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Abstract

People with HIV infection and AIDS requires nutrients that contain macronutrients (carbohydrates, proteins, fats) and micronutrients (vitamins and minerals) in sufficient amount because HIV / AIDS will affect their nutritional status and immune system. HIV infection affect suppress of CD4 T lymphocytes both the quantity and quality. Zinc is known to have a role in increasing CD4 counts in HIV / AIDS patient

This research is experimental with Randomized Pretest Posttest Control Group Design by giving double-blind treatment. The purpose of this study was to analyze the effect of zinc sulphate on CD4 T lymphocytes in HIV / AIDS patients. Population of this study was all outpatients with HIV / AIDS in PIPI units Dr. Soetomo hospital who has earned ARVs. Samples in this study were HIV / AIDS patients from screening results who taken randomly from sub-populations and fulfill inclusion criteria who received ARVs in the last year. Sample of this study divided into two groups, treatment group and control group.

The results of Independent Sample T-test in this study were there are no significant differences CD4 count of respondents between treatment groups who given zinc sulphate supplement and the control group who given a placebo. Most respondents in the treatment group experienced an increase of CD4 count after given an intervention. Results of Chi - Square showing there are a relation between the sample and the difference of CD4 counts.

Keywords: CD4 T lymphocytes; HIV/AIDS patient; Zinc supplementation

Introduction

Unit Perawatan Intermediet dan Penyakit Infeksi (UPIPI) Dr. Soetomo hospital, Surabaya is one place where various components of Governmental Organization (GO) and Non-Governmental Organization (NGO) integrated in order for comprehensive management of people with HIV / AIDS through support, care, and treatment. The numbers of people with HIV / AIDS who came and treated in UPIPI in 2010 are 3.580 people. When compared with data in 2010, the number of people with HIV / AIDS in the mid of 2011 increased by 15.90% to 4.148 people [1].

People with HIV infection and AIDS requires nutrients that contain macronutrients (carbohydrates, proteins, fats) and micronutrients (vitamins and minerals) in sufficient amount because HIV/AIDS will affect their nutritional status and immune system [2-4]. Macronutrient and micronutrient-based nutrients are given in UPIPI. HIV infection affect suppress of CD4 T lymphocytes both the quantity and quality. CD4 count is key to the health of the immune system in patients with HIV / AIDS. The lower the number, the greater the damage, that is caused by HIV [5].

In the nutrition management, supplementation of macro and micro nutrients are important to be optimized. Micronutrient absolutely necessary for people with HIV / AIDS in order to build strong immune system in facing the emergence of opportunistic infections [6,7]. Zinc is a micronutrient that is essential in growth period, before and after birth, as well as to the speed of cellular changes in body tissues such as in immune and digestive system [8]. According to Barasi [9] zinc is important for development of various cellular components in the immune system, particularly T lymphocytes and cells activity that include chemotaxis, phagocytic activity and oxidative bursts.

In a study in Italy with zinc supplementation (200 mg per day) for 1

month can reduce incidence of opportunistic infections, can maintain the stability of weight and increasing CD4 count of AIDS patients who received ARVs therapy. In another study, supplementation with zinc sulphate 0.45 mg / kg / day for 10 weeks can increase CD4 cells significantly [10].

From the studies above, zinc is known to have a role in increasing CD4 counts in HIV / AIDS patient because zinc is a component that can affect various genes for transcription, particularly genes that guide immune system and important for development of non-specific immunity and cell mediated immunity (especially CD4 cell).

This research was conducted to study the effect of zinc sulfate as micronutrient elements and analyzing the number of CD4 T lymphocytes in HIV / AIDS patients. To know changes in number of CD4 T lymphocytes is with look at the number of CD4 T lymphocytes before and after zinc supplementation.

Methods

Study design and population

This research is experimental research with Randomized Pretest Posttest Control Group Design, continuous measurement by giving

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double-blind treatment [10]. Samples in this study were patients with HIV / AIDS who were outpatients aged 22 to 49 years based on screening results who taken randomly from sub-populations and fulfill inclusion criteria. The study sample divided into two groups, treatment group and control group.

Informed consent and ethical clearance

Before data were collected, selected sample was asked to sign an informed consent as evidence of the willingness to being respondents in the study. This study has obtained ethical clearance from the ethical committee Public Health Faculty of Airlangga University.

Supplement

Respondents in this study will give zinc sulphate supplement for treatment group and a placebo for control group.

Dietetic assessment

Information of dietary intake identified using 2x24 hours recall and food record to know energy, protein, fat, carbohydrates, iron, vitamin A, vitamin C and zinc consumption rate of respondents.

Biochemistry assessment

Blood samples that collected by medical analyst conducted to know amount of CD4 T lymphocytes in respondents.

Statistical analysis

The analysis used to test the hypothesis by using Chi-square and Paired T-test. Analysis of CD4 counts before and after zinc supplementation in treatment and control group using Paired T-test, while the analysis of the role of zinc to changes of CD4 T lymphocytes after treatment in treatment and control group using Chi Square.

Results

Characteristics of respondents

Characteristics of respondent who examined, included age and gender. Respondents in this study aged 22 to 49 years. The average age of respondents in the treatment group was 38.85 ± 9.26 years, whereas in control group was 34.85 ± 6.77 years. Statistically, age of the sample was homogeneous. This is evident from the results of independent sample T-test that showed no significant difference age of respondents between treatment and control group ($p < 0.853$).

Family characteristics

Family characteristics include education level, level of knowledge, occupation, family income and family size. Statistical analysis which comparing education level of respondents between two groups showed no significant difference ($p < 0.572$). Similar with knowledge level of respondents, the results of statistical analysis that compares level of knowledge between treatment and control group also showed no significant difference ($p < 0.469$).

Result of statistical analysis that comparing occupation and family income also showed no difference between both groups ($p > 0.05$). Results of statistical analysis comparing number of family members between two groups showed no differences too ($p < 0.846$).

Eating patterns of respondents in both groups include type of food that often consumed by respondents which are staple food + side dish + vegetable and consumption rate in treatment groups were not increased after zinc supplementation.

Nutrition consumption rate of respondents before and after zinc supplementation

Nutrition consumption rate are amount of all kinds of food which consumed by respondents. To determine nutrition consumption rate before intervention was with measured by 2x24 hours recall and converted into energy, protein, carbohydrates and fat, then compared with RDA. As for knowing nutrition consumption rate after supplementation was measured by using a food record or food records book during consume zinc sulphate supplements, then averaged and converted into energy, protein, carbohydrates and fat, then compared with RDA.

Energy, protein, carbohydrates, and fats consumption rate are amount of all kinds of food which consumed and measured by 2x24 hours recall and food record while taking zinc sulphate then compared with RDA, the results are expressed as a percent. Nutrition consumption rate can be categorized as follows: 1) deficit ($< 70\%$ RDA), 2) less ($70-80\%$ RDA), 3) moderate ($\geq 80-99\%$ RDA), and 4) good ($\geq 100\%$ RDA). From results of statistical analysis, energy, protein, carbohydrate, and fat consumption rate in treatment and control group before and after zinc supplementation showed no significant difference ($p > 0.050$).

Differences amount of CD4 T lymphocytes in treatment group and control group

To see the change in CD4 T lymphocytes is with look at CD4 counts before and after zinc sulphate supplementation, and see the difference increase of CD4 count before zinc sulphate supplementation and after zinc sulphate supplementation. Measurement of CD4 T lymphocytes were done through uptake and blood tests which carried out by competent personnel in the morning. Here is a sample distribution according to CD4 T lymphocytes in treatment and control group before and after zinc sulphate supplementation.

The average CD4 counts of respondents in treatment group before and after intervention are lower than control group. However, average CD4 count in treatment group increase after intervention, but decreased in control group. The result of paired T-test shows that there was difference of CD4 count before and after zinc intervention ($p = 0.000$) (Table 1).

After a long period of HIV infection, CD4 counts of HIV / AIDS patients has declined, this is a sign that the immune system is weakened. CD4 T lymphocytes called normal when amount of CD4 count > 1000 cells/mm³, called the condition mild decreases when CD4 count $\geq 500-1000$ cells/mm³, condition moderate decreases when CD4 count $200-500$ cells/mm³ and conditions severe decreases when CD4 count ≤ 200 cells/mm³. Here is a sample distribution according to the category of CD4 count before and after intervention in treatment and the control group.

Σ CD4 Respondents (cell/mm ³)	Treatment Group		Control Group	
	Before	After	Before	After
Average	342,080	414,460	526,770	503,770
Standard Deviation	178,858	222,974	99,081	145,325
Minimum	183	197	331	278
Maximum	780	961	723	849
p-value	0.000			

Table 1: Sample distribution according to CD4 T lymphocytes before and after intervention in treatment group and control group in UPIPI, Dr. Soetomo Hospital Surabaya, 2011.

Category of CD4 Count	Treatment Group				Control Group			
	Before		After		Before		After	
	n	%	n	%	n	%	n	%
Normal (> 1000 cells/mm ³)	0	0,00	0	0,00	0	0,00	0	0,00
Mild Decreases (≥ 500-1000 cells/mm ³)	2	15,30	3	23,00	10	77,00	6	46,10
Moderate Decreases (200-500 cells/mm ³)	10	77,00	10	77,00	3	23,00	7	53,90
Severe Decreases (≤ 200 cells/mm ³)	1	7,70	0	0,00	0	0,00	0	0,00
Total	13	100,00	13	100,00	13	100,00	13	100,00

Table 2: Sample distribution according to the category of CD4 count before and after intervention in treatment group and control group in UPIPI, Dr. Soetomo Hospital Surabaya, 2011.

From Table 2 shows that majority CD4 count of treatment group before intervention in moderate decrease category with 10 people (77.00%) and 1 people (7.70%) in severe decreases category. After intervention, no one respondent in severe decreases category of CD4 count and respondents in mild decrease category increase to 3 people (23.00%).

In control group, before the intervention there are 10 people (77.00%) in mild decrease category of CD4 count and 3 people (23.00%) in moderate decrease category. After the intervention, number of respondents with moderate decrease increased to 7 persons (53.90%) and respondents in mild decrease category decreased to 6 people (46.10%).

Discussion

Role of zinc on the amount of CD4 T lymphocytes

A CD4 test result is the amount of CD4 cells in one cubic millimeter (mm³) of blood. CD4 T lymphocytes called normal when amount of CD4 count > 1000 cells/mm³, called the condition mild decreases when CD4 count ≥ 500-1000 cells/mm³, condition moderate decreases when CD4 count 200-500 cells/mm³ and conditions severe decreases when CD4 count ≤ 200 cells/mm³.

The CD4 count is a key to the health of the immune system. The lower the number, the greater the damage, that is caused by HIV. Zinc sulphate supplement containing 32 mg of zinc ions is given for 3 months with the aim of increasing CD4 T lymphocytes. Increasing of CD4 T lymphocytes can improve the immune of HIV / AIDS respondents.

CD4 cells are a type of white blood cells or lymphocytes. These cells are also called T-4 cells, helper cells or sometimes also called CD4⁺ cells. When HIV infects humans, the cell which most often infects is CD4 cells. HIV becomes part of those cells, and when CD4 cell multiply to fight any infection, they also make more copies of HIV. After a long period of HIV infection, CD4 count decreased. This is a sign that the immune system is damaged [11]. The lower CD4 count, the more likely to be sick.

CD4 receptor on the surface of T-lymphocytes, monocytes, macrophages, Langerhans, dendritic cells, atrosit and microglia. Through internalization process, fusion between membrane of HIV virus with membrane of target cell (CD4 T lymphocytes) happened, resulting cell nucleus of HIV virus enter into cell cytoplasm of T-lymphocytes carrying reserve transcriptase enzyme. After entry into target cells, reserve transcriptase enzyme will use RNA to synthesize

DNA. With the help of integrase enzyme, DNA of HIV virus can integrate into the DNA host, then viral DNA can enter cell nucleus of T-lymphocytes and process of integration happened, transcription, translation of viral proteins and replication of HIV virus. If the amount of viral replication already sufficient, the virus will leave nucleus and host cells to invade CD4 T lymphocytes which uninfected other. In one day HIV able to replicate until reaches 10⁹ – 10¹¹ of new viruses. And so on until there is an imbalance of mutase genes and body need to control through mechanism of DNA repair. When DNA repair are not able to cope, T-lymphocytes that infected must be destroyed through apoptosis. Slowly but sure the number of CD4 T lymphocytes of patients will be depressed and decreased over time. After a long period of HIV infection, CD4 count will decrease. Decrease of CD4 T lymphocytes has resulted in decreased immune system. If immune system more and more damaged, the patient more susceptible to various secondary infection of diseases. In such mechanism, zinc is an essential component, because zinc can prevent integration of viral DNA into the DNA host by binding integrase enzyme through zinc finger protein [12,13].

Differences amount of CD4 T lymphocytes between control and treatment groups

Majority respondents in treatment group experienced increase of CD4 count after given intervention. While respondents in control group majority experienced decreased of CD4 count. CD4 cell count before and after intervention when tested with statistical paired T-test shows there is a significant difference (p < 0,000). This means that zinc interventions affect CD4 count. The difference of CD4 count in the treatment group showed no greater result than the difference of CD4 counts in the control group. This result has different result with Green [14] who stated that no evidence between zinc supplementation and CD4 increase. That is maybe because of the difference between sample sizes in both studies.

Based on observations and interviews to respondents of this study, decrease of CD4 T lymphocytes occurs in respondents who were fasting for Ramadan and perform usual activities and still fasting when their blood taken for checking CD4 T lymphocytes. Despite decrease in CD4 count, respondents did not complain anything even subjectively felt better because the bias fasting while activities as usual.

From various descriptions, researchers concluded that zinc sulphate supplementation in patients with HIV / AIDS who had received ARVs therapy more than one year can increase the number of their CD4 T lymphocytes.

Conclusion

Based on the research that has been done can be summarized as follows:

- I. Majority of respondents aged 30 to 49 years (73.00%) and male (53.85%) with education level is completed high school (69.20%).
- II. The occupation as a trader / self-employed (57.70%), and majority have family income < Rs. 850,000.00 per month with knowledge of nutrition at moderate level.
- III. Energy, protein, fat, carbohydrates, iron, vitamin A, vitamin C and zinc consumption rate before and after zinc intervention in general showed no significant differences (p > α).
- IV. There was a significant increase in the number of CD4 T

lymphocytes in HIV / AIDS patients in UPIPI, Dr. Soetomo Hospital after intervention with zinc sulphate.

- V. There is no difference of CD4 count in treatment and control group, which mean that the difference of CD4 count in treatment group was not greater than the difference in control group.

References

1. Yuyun E (2007) Perbedaan Penambahan Protein Pada Diet Penderita HIV/AIDS Terhadap Perubahan Jumlah Limfosit T-CD4 di Unit Rawat Jalan PIPi RSUD Dr Soetomo. Instalasi Gizi RSDS, Surabaya.
2. Dep Kes RI (2003) Pedoman Nasional Perawatan, Dukungan dan Pengobatan Bagi ODHA: Dirjen Pemberantasan Penyakit Menular and Kesehatan Lingkungan, Jakarta.
3. Firdausi N (2007) Gizi Pada Penderita HIV: Lab/ SMF Ilmu Kesehatan Kulit Dan Kelamin FK Unair / RSU Dr Soetomo, Surabaya.
4. Kemenkes RI (2010) Pedoman Pelayanan Gizi Bagi ODHA: Direktorat Bina Gizi Masyarakat, Ditjen Bina Kesehatan Masyarakat, Jakarta.
5. Yayasan Spiritia (2008) Lembaran Informasi Tentang HIV/AIDS Untuk Orang Yang Hidup Dengan HIV/AIDS (ODHA) Yayasan Spiritia, Jakarta.
6. Nur S (2008) Asuhan Keperawatan Pada Pasien Terinfeksi HIV AIDS: Penerbit Salemba Medika, Jakarta.
7. Yuniastuti (2005) Infeksi Oportunistik Pada AIDS. Pokdisus AIDS FKUI, Perhimpunan Dokter Peduli AIDS Indonesia, Balai Penerbit FKUI, Jakarta.
8. Adriani (2009) M Pengaruh Seng Pada Suplemen Vitamin A Dosis Tinggi Terhadap Status Infeksi dan Pertumbuhan Linier Balita Disertasi, Universitas Airlangga, Surabaya.
9. Barasi M (2007) Nutrition at a Glance. Wiley-Blackwell, New Jersey.
10. Patrick L (2000) Nutrients and HIV: Part 2 - Vitamins A and E, Zinc, B-vitamins, and Magnesium. *Altern Med Rev* 5: 39-51.
11. Baratawidjaja, Karmen G (2004) *Imunologi Dasar*. Edisi Ke-6, Jakarta, FKUI.
12. Nasronudin (2007) HIV and AIDS Pendekatan Biologi Molekuler, Klinis, dan Sosial. Airlangga University Press, Surabaya.
13. Nasronudin, Hadi, Vitanata, et al. (2007) *Penyakit Infeksi Di Indonesia*. Airlangga University Press, Surabaya.
14. Green JA, Lewin SR, Wightman F, Lee M, Ravindran S (2005) A Randomized Controlled Trial of Oral Zinc on the Immune Response to Tuberculosis in HIV-Infected Patients. *Int J Tuberc Lung Dis* 9: 1378-1384.

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