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Original Article

Zinc supplementation and cellular immune response in splenectomized thalassemia major

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Abstract

Background Thalassemia patients are generally immunocompromised, making them susceptible to infection. A study at the Thalassemia Center at Dr. Cipto Mangunkusumo Hospital, Jakarta, showed that all thalassemia patients experienced zinc deficiency. Decreased cellular immune response has been associated with zinc deficiency, and splenectomy exacerbates the risk of infection.

Objective To evaluate for improvement of cellular immune response in splenectomized thalassemia major patients after zinc supplementation.

Methods This randomized, double-blind, controlled trial was conducted on splenectomized thalassemia major patients over a 12-week period. The inclusion criteria were splenectomized thalassemia major patients aged > 12 to 18 years with negative HIV test results. Patients receiving corticosteroids were excluded. Fifty-six subjects were randomly divided into two groups, the zinc group and the placebo group. Before and after the 12 -week treatment, we evaluated subjects' serum zinc, T lymphocyte count, CD4+ T lymphocyte count, CD8+ T lymphocyte count, and CD4+/CD8+ ratio and were analyzed with unpaired T-test, Mann Whitney test, and Wilcoxon Signed Rank test.

Results After zinc supplementation, serum zinc serum levels in 18/28 subjects in the zinc group became normalized. None of the cellular immune response parameters were significantly different between the two groups after zinc supplementation ($P > 0.05$). This finding might have been due to the subjects' compliance which was lower in the zinc group (75.82%) than in the placebo group (83.19%).

Conclusion Cellular immune response in splenectomized thalassemia major patients is not significantly changed after 12 weeks of zinc supplementation. **[Paediatr Indones. 2024;64:145-51; DOI: 10.14238/pi64.2.2024.145-51**].

Keywords: *cellular immune response; zinc supplementation; thalassemia major*

Thalassemia is a hereditary anemia of a single gene mutation causing defects in globin production.[1] Infection is a major cause of morbidity and mortality in thalassemia patients worldwide.[2-4] Thalassemia patients are susceptible to infection due to their suppressed immune response. Such defective immune responses may be due to iron overload, splenectomy,[3,5-7] and/or zinc deficiency.[3,7] Iron overload is the leading cause of immune disorders in thalassemia patients. Studies of innate immunity in thalassemia patients show impaired macrophage phagocytosis function,[3,5] whereas studies of adaptive (cellular) immunity show increased number and activity of CD8+ suppressor cells, decreased CD4+/CD8+ ratio, decreased T lymphocyte proliferation, and increased T lymphocyte activation.[3,5]

In thalassemia patients, the spleen has an important function in immune response because it is a source of immunocompetent lymphocytes.

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After splenectomy, production of CD4+-mediated antibodies against antigens is impaired.[5] The indications of surgical splenectomy in thalassemia patients are hypersplenism, blood transfusion requirements exceeding 200-220 mL/kg/year and massive splenomegaly accompanied by upper left quadrant abdominal pain. Surgical splenectomy is recommended in thalassemia patients who are above 5 years old in order to prevent susceptibility to infection.[8,9]

Zinc is needed to induce the biological activity of thymulin, a hormone produced by the thymus. Zinc deficiency leads to atrophy of the thymus and lymphoid tissue, impaired formation of cytotoxic T lymphocytes, decreased NK cell activity, impaired reactivity of delayed-type skin test, decreased lymphocyte proliferation response to mitogens, and decreased number of selective CD4+ cells.[10,11] A study in 2006 shows that all thalassemia patients at the Thalassemia Center, Cipto Mangunkusumo Hospital, had low zinc levels (<75 υg/dL).[11] Serum zinc level in thalassemia patients is not related to zinc intake from food, but is more affected by ferritin level. Increased ferritin levels in thalassemia can inhibit zinc absorption because iron and zinc compete for binding to transferrin after being absorbed in the jejunal and ileal mucosa.[12]

Our aim was to evaluate the immune response in splenectomized thalassemia major patients, specifically, total lymphocyte count, CD4+ T lymphocyte count, CD8+ T lymphocyte count, and CD4+/CD8+ ratio, before and after zinc supplementation.

Methods

A randomized, double-blind, controlled trial was conducted in splenectomized thalassemia major patients at the Thalassemia Center, Cipto Mangunkusumo Hospital, Jakarta. Inclusion criteria were children aged > 12 to 18 years, had negative HIV test result, were willing to participate in the study, and provided written informed consent. Patients receiving corticosteroid and tuberculosis therapy were excluded. Subjects were recruited by total population sampling and were randomized into two groups, zinc and placebo. The zinc group received zinc sulfate syrup at a dose of 1.5 mg/kg/day, with a maximum dose of 50 mg/day, while the placebo group received sucrose syrup in the same form, color, and taste as the zinc sulfate syrup, for 12 weeks. Medication was prepared by the hospital pharmacy; participants and authors were blinded to the contents of the supplement. At the beginning of the study, subjects underwent history-taking including the food recall, physical examination, and laboratory examinations, including peripheral blood, ferritin, transferrin, and transferrin saturation. Measurements of serum zinc, T lymphocyte count, CD4+ T lymphocyte count, CD8+ T lymphocyte count, and CD4+/CD8+ ratio were taken before and after 12 weeks of zinc/placebo treatment. Serum zinc was examined using inductively coupled plasma mass spectrometry, while flow cytometry was used to count the various lymphocytes.

Normal serum zinc level was 25-148 ug/L for children aged 10-14 years and 46-130 ug/L for children aged 14-18 years. Improved immune response was defined as an increase in T lymphocyte count, an increase in CD4+ T lymphocyte count, a decrease in CD8+ T lymphocyte count, and an increase in CD4+/CD8+ ratio after zinc supplementation in the zinc group.

All parameters were expressed as mean and standard deviation (SD). Statistical analysis was performed using *SPSS version 20* software. Changes in immunological parameters in both groups were analyzed using unpaired T-test for normally distributed variables and Mann-Whitney test for non-normally distributed variables. This study was approved by the Research Ethics Committee of Universitas Indonesia.

Results

Fifty-eight patients met the inclusion criteria, but one patient from the zinc group passed away and one patient from the placebo group received corticosteroids during the study (**Figure 1**). The initial characteristics of subjects are shown in **Table 1**.

The effect of supplementation was assessed through the absolute changes. It was calculated by subtracting after supplementation value from before supplementation value. Serum zinc levels in both groups before and after supplementation are shown in **Table 2**.

There was no significant difference in serum zinc

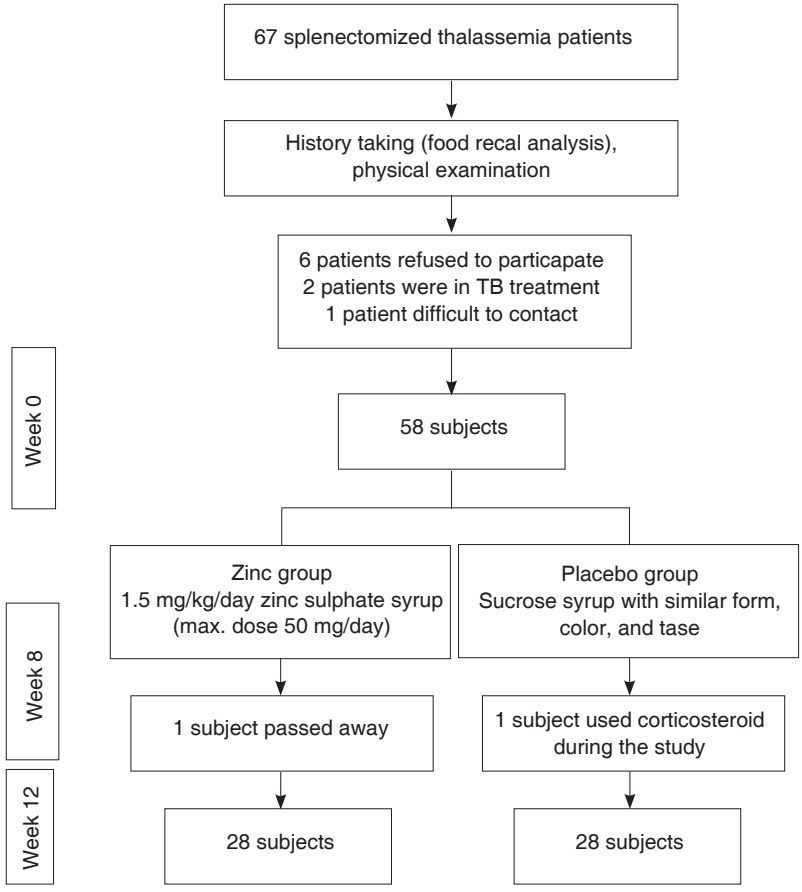

Figure 1. Recruitment of splenectomized thalassemia major subjects

levels between the two groups before supplementation, but serum zinc levels significantly increased in both groups after supplementation (P<0.001 for both groups). Increased serum zinc levels after supplementation was absolutely higher in the zinc group than in the placebo group, as it stated in absolute change (P<0.001). Treatment compliance was not significantly different between groups [83.19 (SD 14.41)% in the placebo group and 75.82 (SD 15.05)% in the zinc group (95%CI -0.523 to 15.266; P=0.067)].

There was a decrease of median T-lymphocyte count after supplementation in both group. However the data is not normally distributed, thus the median value was calculated. We found the median value was -666,5 (-3,911 - 1,145) in zinc group and -675,5 (-34,302 - 1,770) in placebo group (**Table 3**). Therefore, there was no significant difference between T-lymphocyte count before and after supplementation in two groups.

There was a significant decrease in CD4+ T lymphocyte count in both groups after supplementation. The absolute changes (median value) were -196 (range -865 -579) in zinc group and -169 (range -17,953 - 234) in placebo group which indicated as there was a decrease in median CD4+ T-lymphocyte count after supplementation (**Table 4**). This study did not find any significant change in CD4+ T lymphocytes count after zinc supplementation in both groups.

The number of CD8+ T lymphocytes decreased in both groups, however statical significance was only observed in the zinc group. The absolute change (median value) indicated negative values as there was a decrease in median CD8+ T-lymphocyte count after supplementation compared to median value before supplementation. There was no significant change in both zinc and placebo groups. Thus,

Table 1. Characteristics of subjects prior to treatment

Characteristics	(N=56)
Type of thalassemia, n (%)	
Beta	29 (51.8)
Beta-HbE	27 (48.2)
Alpha	0
Nutritional status, n (%)	
Obese	0
Overweight	0
Normal	25 (44.6)
Underweight	20 (35.7)
Wasting	11 (19.6)
Blood transfusion frequency, n (%)	
Rare (< 2 times/year)	1 (1.8)
Sometimes (3-4 times/year)	7 (12.5)
Often (> 4 times/year)	48 (85.7)
Type of blood, n (%)	
PRC	4 (7.1)
Leuko-depleted PRC	21 (37.5)
Washed PR	31 (55.4)
Median ferritin (range), ng/mL	6,585 (645-21,835)
Median transferrin saturation (range), %	100 (26-118)
Iron chelation agent	
Deferoxamine (DFO)	7 (12.5)
Deferiprone (DFP)	32 (57.1)
Deferasirox (DFX)	12 (21.4)
Combination DFO + DFP	5 (8.9)
Hepatitis marker	
Hepatitis B	13 (23.2)
Hepatitis C	22 (39.3)

PRC=packed red cell

zinc supplementation did not affect the CD8+ T lymphocytes count (**Table 5**).

The CD4+/CD8+ ratio increased in the zinc group and decreased in the placebo group, but neither change was statistically significant. Absolute change (mean value) in CD4+/CD8+ ratios was not significantly in both groups after zinc supplementation (**Table 6**).

Discussion

Patients with thalassemia major in Indonesia have unique characteristics, as they commonly have transfusion problems and inadequate use of iron chelation. Transfusions are often conducted out of schedule as patient families lack the financial resources to receive care in the hospital. In addition, poor compliance in consuming iron chelation treatment leads to excessive iron levels. The state of iron overload is measured by ferritin level and transferrin saturation. Excessive iron in splenectomized subjects impacts their immune systems, making them susceptible to infection. Thalassemia major patients with a history of splenectomy and aged > 10 years commonly suffer from severe bacterial infections.[13]

Table 2. Analysis of zinc supplementation and serum zinc levels in splenectomized thalassemia major subjects

Variables	Serum zinc level		P value
	Zinc group (n=28)	Placebo group (n=28)	
Initial mean serum zinc (SD), µg/L	44.79 (9.22)	48.14 (8.31)	0.158¶
Final mean serum zinc (SD), µg/L	75.04 (26.60)	56.32 (11.99)	
P value*	< 0.001	< 0.001	
Mean value change µg/L	30.25 (27.80)	8.18 (9.12)	<0.001*

*unpaired T-test; ¶Mann-Whitney test; initial=mean serum zinc level before zinc supplementation; final=mean serum zinc level after zinc supplementation

Table 3. Analysis of zinc supplementation and T lymphocyte count in splenectomized thalassemia major subjects

Variables	T lymphocyte count		P value
	Zinc group (n=28)	Placebo group (n=28)	
Median T-lymphocyte count at start (range), cells/uL	4,360.5 (1,855 - 11,010)	4,329 (1,288 - 45,699)	0.575¶
Median T-lymphocyte count at end (range), cells/uL	4,430.50 (1,570 - 8,311)	3,811 (1,292 -11,397)	
P value¥	0.001	<0.001	
Median value change (range), cells/uL	- 666.5 (-3,911 - 1,145)	- 675.5 (-34,302 - 1,770)	0.000¶

¶Saphiro-Wilk test; ¥Wilcoxon signed rank test

Table 4. Analysis of zinc supplementation and CD4+ T lymphocyte counts in splenectomized thalassemia major subjects

Variables	CD4+ T lymphocyte count		P value
	Zinc group (n=28)	Placebo group (n=28)	
Median CD4+ T-lymphocyte count at start (range), cells/uL	1,940.50 (739 - 4,500)	1,711 (363 - 21,177)	0.367¶
Median T-lymphocyte count at end (range), cells/uL	1,786 (762 - 4,248)	1,622 (481 - 3,224)	
P value¥	0.049	0.003	
Median value change (range), cells /uL	-196 (-865 - 579)	-169 (-17,953 - 234)	0.629¶

¶Saphiro-Wilk test; ¥Wilcoxon signed rank test

Table 5. Analysis of zinc supplementation and CD8+ T lymphocyte count in splenectomized thalassemia major subjects

Variables	CD8+ T lymphocyte count		P value
	Zinc group (n=28)	Placebo group (n=28)	
Median CD8+ T-lymphocyte count at start (range), cells/uL	1.974 (675 - 5,038)	1.655 (652 - 4,704)	0.446¶
Median CD8+ T-lymphocyte count at end (range), cells/uL	1,940 (431 - 3,816)	1,364 (545 - 6,943)	
\Nilai P¥?	0.040	0.116	
Median value change (range), cells/uL	- 212 (-1,324 - 706)	-62.5 (-1,938 - 2,239)	0.781¶

¶Saphiro-Wilk test; ¥Wilcoxon signed rank test

Table 6. Analysis of zinc supplementation and CD4+/CD8+ ratio in splenectomized thalassemia major subjects

Variables	CD4+/CD8+ ratio		P value
	Zinc group (n=28)	Placebo group (n=28)	
Median CD4+/CD8+ ratio at start (range)	0.94 (0.4 - 2.1)	1.0 (0.3 - 2.0)	0.901¶
Median CD4+/CD8+ ratio at end (range)	1.02 (0.4 - 2.1)	0.913 (0.4 - 1.9)	
P value¥	0.175	0.149	
Mean value change (SD)	0.05 (0.21)	-0.06 (0.20)	0.058*

*Unpaired T-test; ¶Mann-Whitney test; ¥Wilcoxon signed rank test

The majority of our subjects had low zinc serum levels prior to treatment. Measure zinc intake by conducting history taking (food recall) and calculating the amount of intake both in absolute and relative amounts (%) per day, found that all thalassemia patients consumed low zinc intake. The cause of low zinc levels in thalassemia is a lack of zinc in daily food intake. Other causes are hyperzincuria due to hemolysis, impaired zinc metabolism, high excretion of zinc in sweat, and malnutrition.[14] Healthy men who were given zinc at a dose of 50 mg/day and vitamin E at a dose of 400 mg/day had significant increases in zinc and vitamin E levels.[15] Moreover, zinc supplementation in thalassemia patients showed an improvement in subjects' oxidative stress state through the reduction of antioxidant enzyme levels ($P<0.029$).[16] We noted significantly increased serum zinc levels in the zinc group. However, zinc levels also increased 17.75% in the placebo group, possibly due to increased zinc intake because of education provided by the dietary nutritionist. This condition was also found in a previous study reported an increase in zinc levels not exceeding 20% above the baseline.[15]

The zinc group received zinc at 1.5 mg/kg/day, with a maximum dose of 50 mg. Such zinc supplementation for 12 weeks succeeded in increasing zinc levels to normal in only 18 of 28 subjects. Zinc absorption follows a saturable, dose-response model. Serum zinc increases 2-5 days after zinc supplementation intake, it remains stable during supplementation use, and then decreases to the baseline value in 14 days after discontinuation. Serum zinc absorption will not increase if zinc intake has reached ~25-30 mg/day.[17] A study on the absorption

of zinc solution in eight healthy adults found a dose of 13 mg zinc to be the maximum amount that could be absorbed, regardless of the amount taken. When given more than 20 mg of zinc supplementation it may result the reduction of zinc absorption.[18] Hence, the proper dose of zinc supplementation for thalassemia patients needs further study.

We found that after zinc supplementation, the number of T lymphocytes increased slightly, but the number of CD4+ and CD8+ T lymphocytes decreased, resulting in the CD4+/CD8+ ratio remained constant. Lack of substantial effect on immune response after zinc supplementation may have been due to the duration of administration or an inappropriate dose of zinc supplementation. The state of chronic zinc deficiency in splenectomized thalassemia major subjects may also have required an increased duration of zinc supplementation for this study. Zinc administration for 12 weeks may not have been long enough to increase serum zinc levels in our thalassemia major patients.

Compliance in consuming supplementation was lower in the zinc group than in the placebo group, possibly resulting in a lower zinc dose in those who did not fully comply. An increase to normal zinc levels only occurred in 18 of 28 subjects in the zinc group. Therefore, an effect of zinc supplementation in improving cellular immune response needs further investigation.

Another reason for the lack of immune response improvement was a deficiency of antioxidant vitamins and other trace elements, such as selenium, in thalassemia patients. Vitamins and trace elements can protect tissues from damage by reactive oxygen by regulating transcription factors as well as producing cytokines and prostaglandins. In addition, they can induce immune responses through Th1 cytokines and prevent Th1 to Th2 transfer so that the immune response improves.[19]

We recommend that zinc supplementation be given to splenectomized thalassemia major patients, not only for improving the immune response, but also for other enzyme functions. Improving the immune response may require not only zinc but also vitamins and other trace elements. Recent study on impacts of zinc supplementation on monocyte function stated that zinc supplementation increased the proportion of monocyte after administration of 30 mg of elemental zinc per day for eight weeks.[20] Thus, the study aligns with our recommendation that zinc supplementation does improve immune function.

There was no improvement in specific immune responses (increased T lymphocyte count, increased CD4+ T lymphocyte count, decreased CD8+ T lymphocyte count, and increased CD4+/CD8+ ratio) between splenectomized major thalassemia patients in the zinc group and the placebo group in our 12-weeks study. Further study is needed regarding the appropriate dose and duration of zinc supplementation to improve the immune response of splenectomized thalassemia major patients.

Conflict of interest

None declared.

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