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The correlation between zinc and monocyte phagocytosis in patients with major b-thalassemia

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Abstract

Background: Zinc depletion decreases monocyte functions and survival while excessive amount of zinc inhibits monocyte activation. Monocytes shift from conducting intercellular communication to becoming innate immune function as a response. This study aims to examine the influence of zinc status on the monocyte phagocytosis in patients with major beta-thalassemia.

Materials and Methods: This study was a randomized-placebo-controlled trial. The patients were randomly assigned into either the zinc-treated group using zinc gluconate 50mg daily or the placebo group. Analysis is based on the 12-weeks observation of the complete blood count, plasma zinc level, and phagocytosis level of monocytes. The phagocytic activity of monocytes was measured using atomic absorption spectroscopy (AAS) or x-ray fluorescence (XRF). The comparisons of the data within each group were analyzed using Mann-Whitney test.

Results: The results indicated no significant differences in patients' characteristics; the level of plasma zinc at week 12 in the zinc-treated group (67.41+14.4) was significantly higher than the placebo group (54.37+9.38) (p=0.047). The phagocytosis levels of monocyte at week 12 in zinc-treated group (8.70+4.61) were higher than the placebo groups (8.23+4.22) (p=0.002). The ferritin level of zinc-treated group was higher than placebo group (p=0.084), while high level of ferritin is associated with higher level of monocyte phagocytic activity, the result is statistically significant (p=0.002). The results also showed that higher level of plasma zinc insignificantly correlates with lower phagocytic activity of the monocytes (p=0.059).

Conclusion: The immune mechanisms in response to zinc-deficient environment underlying the shifting between adaptive to innate immune response involves multiple molecular components of the immune system and have been attributed to specific features of □-thalassemia, in which overall immune activity is decreased even though the phagocytic activity of monocytes is increased.

Keywords: Major beta thalassemia, Monocytes, Phagocytosis, Zinc

Introduction

Zinc has been known to play an important role in the immune system, affecting both innate and adaptive immune cells (1). Zinc influences specific cellular subsets of the immune system including proliferation and function (3). The innate immune system as the first line of defense has been proven to be weakened by zinc deficiency (4). Zinc deficiency also affects monocytes, in which case, zinc can have both positive and negative influences (4). On one hand, zinc depletion decreases monocyte functions and survival, but on the other hand, excessive amount of zinc inhibits monocyte activation (5,6). Monocytes are responsible for cytokine production, and the release of inflammatory cytokines (7). The production of pro-inflammatory cytokines uses intracellular zinc signals as a response to lipopolysaccharide (LPS) consisted in the negative bacteria's cell walls. Zinc supplementation also enhances the release of cytokines in peripheral mononuclear blood cells (PBMC) (5,8-9). In addition, secretion of pro-inflammatory cytokines can be stimulated directly by high extracellular Zn^{2+} incubation; on the other hand, high extracellular zinc concentrations have been reported to inhibit LPS-induced

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monokine production (9). The recent study revealed that zinc supplementation results in increased proportion of monocytes expressing transmembrane TNF-alpha (6). In zinc-deficient environment, the immune system goes through reprogramming that leads to a 'shift' from adaptive to innate immune defense, in which monocytes shift from intercellular communication to basic innate defensive functions as a response (10). This phenomenon increases apoptosis of lymphocytes, especially T cells; while myeloid cells such as neutrophils and monocytes are not affected. In some cases, the increase of myeloid cells occurs both in the bone marrow and peripheral circulation. This phenomenon occurs due to the fact that immune cells production is heavy when there is scarce presence of essential nutrients, thus, the system sets priorities in which case it channels resources toward the first line of immune defense (10). In the case of *in vitro* zinc deficiency, phagocytosis and cytotoxicity increase alongside an enhanced oxidative burst, accompanied by increase maturation into macrophages, proving that low zinc status promotes differentiation of monocytes, however, there is an impairment in activation of the cells. Thus, this occurrence supports the shifting theory of monocytes even further, displaying the occurrence of shifting from intercellular communication via cytokines to concentrating the cellular activity on direct anti-pathogenic functions (11,12). This shift is beneficial in decreasing requirement for communication between innate and adaptive immune cells by cytokines in which condition that the monocytes focus on phagocytosis and oxidative burst during deficiency, that is why zinc deficiency is proposed to have positive effects on the increase of monocyte phagocytosis (3, 10-12). Beta (β) thalassemia is an autosomal recessive disease resulted from gene mutations that affects the synthesis of globin chains. This mutation results in chronic hemolytic anemia from the first year of life. Zinc deficiency is a common feature in β-thalassemia, which highly related to immune deficiency as explained above, especially on its role as an immune regulator. A comprehensive study by Haase *et al* (2007) investigated the effects of zinc on different leukocyte subsets through the use of microarray technology to analyze and compare the changes in mRNA expression in cell culture models of monocytes. The study revealed that zinc affects entire functional networks of genes that are related to pro-inflammatory cytokines and cellular survival as well as an extensive impact on leukocyte gene expression, but, there was no common protein (e.g. transcription factor or signaling protein) that mediates the immunological effects of zinc. To be clear, no remarkable effects on gene expression were found despite the extensive impacts on leukocyte gene expression, as there was no fundamental protein production affected. However, it is likely that zinc interacts with the gene expression by regulating signaling pathways through post-translational events. There was still possibility that zinc has significant – not necessarily fundamental – impacts on leukocyte subsets, including monocytes in β-thalassemia (13). Therefore, this study aims to examine the influence of zinc status on monocyte phagocytosis in patients with major β-thalassemia.

Materials and Methods

Patients

The target population of this study consisted of β-thalassemia patients who had undergone splenectomy and received treatments at Hematology-Oncology Division, Pediatric Department RSCM from January 2014 to December 2014. A total of 58 β-thalassemia patients who had undergone splenectomy were recruited for the study. Thalassemia was defined and diagnosed based on National Practice Guideline for Thalassemia in Indonesia Year 2014. All 58 patients who had received information before participating in

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the study gave their written informed consent on the experimental procedures. This study was approved by Ethics Committee for Researches involving Human Subjects from the Faculty of Medicine, Universitas Indonesia in Jakarta (Ethical code: 260/H2.F1/ETIK/2013). The inclusion criteria include: 1) β-thalassemia patients who had undergone splenectomy, 2) age 15-35 years old, 3) being treated at Hematology-Oncology Division, Pediatric Department, Cipto Mangunkusumo General Hospital (RSCM), 4) agreed to be enrolled in the study; while the exclusion criteria was consisted of: 1) incomplete medical record, 2) refusal to be enrolled in the study by the patients, 3) restricted drug consumptions, 4) restricted habits (e.g. smoking).

Sample size

The sample was acquired in consecutive manner, in which the patients were enrolled in the study when they partake in consultations in respective policlinic and they have fulfilled the inclusion criteria. The estimation of sample size was counted using sample size prediction based on categorical analysis which resulted in 57.78 patients ~ 58 patients which were divided evenly into 29 subjects in the group with zinc supplementation and 29 subjects in the placebo group.

Study design

This study was a randomized placebo-controlled trial. The patients were randomly assigned into either the intervention group or the placebo group. The patients in the zinc-treated group were given a dose of zinc gluconate 50 mg daily while the patients in the placebo group received placebo (50 mg of corn starch daik). The patients in both groups were assigned to take 1 capsule per day for 12 weeks. At the beginning and the end of the study, blood samples were collected for analysis of complete blood count, plasma zinc level, and phagocytosis level of monocytes. The phagocytic activity of monocytes was measured using atomic absorption spectroscopy (AAS) or x-ray fluorescence (XRF).

Evaluation of monocyte phagocytic activity

AAS is a spectro-analytical procedure for quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. This technique provides valuable information on concentration required elements present in the sample. XRF is the emission of characteristic secondary or fluorescent x-rays from a material that has been excited by bombarding high energy x-rays or gamma rays. XRF is a powerful quantitative and qualitative analytical tool for elemental analysis of materials. It is an established method that is just gaining popularity as a practical and portable technique due to rapid, multi-element measurements with minimal sample preparation. The method is fast, accurate, non-destructive and is based on measuring x-rays emitted from the elements in a sample on irradiation with higher energy x-rays.

Statistical analysis

The data are presented as the mean+standard deviation (SD). A Chi-square was performed to see if the data has a normal distribution (parametric). An independent sample t-test was performed to acquire mean and SD. The comparison of the data between groups were analyzed using Mann-Whitney test. Statistical significance was considered for those with a p-value less than 0.05.

Results

The characteristics of the thalassemia patients are presented below on Table I. At baseline, our results showed no significant differences, including age, body mass index (BMI), hemoglobin levels, zinc plasma levels, ferritin levels, compliance levels, and transfusion frequency between the placebo and zinc-treated groups. The results showed that the level of plasma zinc in the zinc-treated group experienced a hike from baseline ($p<0.05$) and is significantly higher than the placebo group ($p<0.05$)

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(Table II). Within the groups, the plasma zinc level in placebo group was also increased after 12 weeks of placebo supplementation, so do the subjects in the zinc-treated group after 12 weeks. There are a lot other factors that cause such things to take place, which will be discussed further in the Discussion. The results also show that zinc supplementation had significant effects on the zinc-treated group when compared to placebo group after 12 weeks of the study ($p<0.05$). As also seen in Table II, the phagocytosis levels of monocyte in zinc-treated group are higher than the placebo groups ($p=0.037$). Table III presents the correlation between ferritin levels and the monocyte phagocytic activity. In which the ferritin levels are divided into low and high based on the subjects scoring to see whether the high and low levels of ferritin affect the phagocytic activity of the monocytes. As seen in Table 3, high level of ferritin is associated with higher level of monocyte phagocytic activity, the result is statistically significant ($p=0.002$). The plasma zinc level was also divided into two groups: low and high. Lower level of plasma zinc correlates with higher level of monocyte phagocytic activity. On the other hand, higher level of plasma zinc correlates with lower phagocytic activity of the monocytes, although not statistically significant ($p=0.059$), see Table IV.

Table I: *Characteristics of subjects.*

Aspects	Placebo group (n=29)	Zinc-treated group (n=29)
Age (years)	26.92±9.93	25.89±6.90
Body mass index (kg/m²)	17.41±2.81	18.44±2.64
Hemoglobin levels (g/dL)	8.29±1.99	7.91±0.81
Zinc plasma (µg/L)	47.34±12.34	44.48±26.48
Compliance (%)	78.82±36.02	83.03±30.73
Transfusion frequency (x/year)	8.62±4.62	7.62±4.62
Ferritin levels (ng/dL)	6609.02±5061	7649.83±6551.80

Table II: The effects of zinc supplementation on the plasma zinc levels, ferritin levels, and monocyte phagocytosis after 12 weeks.

Parameter	Placebo group (n=29)		Zinc-treated group (n=29)		P-value
	Baseline	Week 12	Baseline	Week 12	
Plasma zinc level (µg/L)	47.34±16.35	54.37±9.38	44.48±12.5	67.41±14.4	0.047
Ferritin levels (ng/dL)	6609.02±5061	6772±48809.2	7649.83±6551.80	8512.52±2029.6	0.084
Monocyte phagocytosis levels (%)	8.23±5.32	8.23±4.22	5.43±3.05	8.70±4.61	0.037

Table III: The correlation between varied ferritin levels and monocyte phagocytic activity.

	Ferritin Levels		
	Low	**High**	**P Value**
Monocyte phagocytosis (%)	2.7 ± 1.99	6.8 ± 5.58	**0,002**

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Table IV: The correlation between varied plasma zinc levels and monocyte phagocytic activity.

	Plasma Zinc Levels		
	Low	**High**	**P value**
Monocyte phagocytosis (%)	7.59 ± 6.00	. 6.46 ± 5.14	**0,059**

Discussion

This study aimed to investigate the possibility of zinc's role in the modulation of immune function in patients with β-thalassemia. As expected, the baseline plasma zinc level of the subjects enrolled in this study were reckoned to be rather low compared to those in healthy individuals. Deprived zinc status is highly associated with weaker immune system (4). Sustaining plasma zinc levels within the normal limit could be achieved with zinc intake that may be beneficial in enhancing zinc function and thus avoiding complications that might occur due to this condition (10).

This study established that 12-weeks zinc supplementation resulted in improved plasma zinc levels and ferritin levels. However, there are slightly different links of association between the effects of ferritin levels on monocyte phagocytic activity and the effects of plasma zinc levels on monocyte phagocytic activity. The level of monocyte phagocytic activity is directly proportional to the ferritin levels while it is inversely proportional to plasma zinc levels. When the ferritin level is higher, the level of monocyte phagocytic activity is also higher. On the other hand, when the ferritin level is lower, the level of monocyte phagocytic activity is also lower.

The result is in accordance with a study by Mayer (2014), whereas it was found that in response to deficiency, the monocytes shift from intercellular communication to basic innate defensive functions, which explained the increased level of phagocytic activity during zinc deficiency (3). On the contrary, a study by Hong Gao (2018), stated that children who received 10-20mg of zinc supplementation per day had proven to have improved monocyte phagocytic capacity (4). On the other hand, another study by Prasad (2009) stated that reducing zinc concentration in peripheral blood by treating the cells with TPEN or by removing zinc from the culture medium using chelator Chelex100, the level of innate function is increased significantly including phagocytic activity (4). However, this particular study has different study design which is *in vitro* study, hence, the results is not in accordance with the results of RCTs.

The most recent study on the impacts of zinc supplementation on monocyte function disclosed that zinc supplementation increased the proportion of monocyte that expressed transmembrane TNF-alpha after administration of 30 mg of elemental zinc per day for eight weeks. This supports the assumption that zinc supplementation does improve immune function of the monocytes (6). In another study by Sheikh (2010), zinc-treated children had lower monocyte phagocytic activity as well as lower oxidative burst capacity when compared to control group (7).

Some of the previous studies believed that this phenomenon is caused by 'shifting' that occurs during zinc deficiency, in which case, the immune system undergoes reprogramming that converts adaptive to innate immune defense (10). The studies also suggest that low zinc status promotes mediation of host defense via phagocytosis and oxidative burst (7-10). The shifting allows cellular activity on direct anti-pathogenic functions without intercellular communication via cytokines. Within such condition, monocytes focus on phagocytosis and oxidative burst. Thus, it

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can be concluded that low zinc status promotes greater monocyte phagocytic activity (3,7-10).

Conclusion

Immune deficiency constitutes an important part of the clinical spectrum of β-thalassemia, being highly linked to significant morbidity and mortality. The recently recognized immune mechanisms in response to zinc-deficient environment underlying the shifting between adaptive to innate immune response involves multiple molecular components of the immune system and have been attributed to specific features of β-thalassemia, in which overall immune activity is decreased even though the phagocytic activity of monocytes is increased. Zinc supplementation for patients with β-thalassemia is crucial due to the fact that phagocytic activity and oxidative burst of monocytes are not sufficient to prevent infections. Additional studies are required to establish clinical significance of the suspected precipitating mechanisms, hence providing new alternative to further ameliorate the survival rate and quality life of the patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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