ORIGINAL ARTICLE

Correlation of Epidermal Fibroblast Growth Factor and Clinical Improvement of Asthma in Children after Zinc Supplementation

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ARTICLE INFO

Article history:
Received 27 August 2020
Received in revised form 04 September 2020
Accepted 09 September 2020
Available online 30 September 2020

Keywords:
Asthma,
Children,
Zinc,
Epidermalfibroblast growth factor.

ABSTRACT

Background: Background The presence of remodeling process on the pathogenesis of asthma that involves some growth factors such as epidermal growth factor (EGF) and fibroblast growth factor (FGF) causes the chronicity of the disease. The role of zinc on the pathogenesis of asthma is being widely investigated. This study aimed to analyze the correlation between EGF and FGF2 and clinical improvement of asthma after zinc supplementation.

Methods: A quasi-experimental study was conducted in Outpatient Clinic Dr. Soetomo Hospital. The samples were persistent asthma patients from 6-15 years old who received controller therapy. The samples were divided into 2 groups, those who received zinc supplementation as the intervention group, and who received pacebo as the control. EGF and FGF2 plasma level of both groups were measured, and clinical improvement was evaluated with Childhood Asthma Control Test (C-ACT).

Results: There were 11 patients who received zinc supplementation and 12 patients in the control group. There was a significant difference ($p = 0.000$) on the increase of EGF level in the intervention group (55.59 ± 6.48) than the control (5.35 ± 5.55). There was a significant difference ($p = 0.000$) on the increase of the FGF2 level in the intervention group (6.37 ± 1.41) than the control (0.72 ± 0.48). The increase of EGF ($r = 0.592$; $p = 0.003$) and FGF2 ($r = 0.607$; $p = 0.002$) would be followed by the increase of C-ACT scores.

Conclusion: Zinc supplementation increase EGF and FGF2 levels. This improvement is correlated with clinical improvement of patients.

INTRODUCTION

Asthma is a chronic inflammatory disease that can affect children and adults. It is characterized by hyperactivity of respiratory tract, airway obstruction, and chronic inflammation.[1] The pathogenesis of asthma is complex. The role of adaptive immunity and antigen-specific Th2 cells was first identified in the pathogenesis of asthma, and then the innate lymphoid cells type 2 (ILC2s) that produce type-2 cytokines such as IL-4, IL-5, and IL-13 were also known to have a role in the pathogenesis of asthma. Moreover, the airway remodeling process that involves growth factors such as epidermal growth factor (EGF) and fibroblastgrowth factor (FGF) contribute to the chronicity of asthma. Although the treatment of asthma has been improved, the morbidity of the disease and the response to the treatment are various. It depends on multifactorial aspects such as genetic, environmental,economic, and nutritional factors. One of the nutritional factors that is suspected to play a role in the pathogenesis of asthma is zinc (Zn).[2,3] The roles of zinc supplementation in the pathogenesis of asthma are anti- inflammatory, anti-oxidant, and anti-apoptosis agents.[4,5] The purpose of this study was to analyze the correlation between EGF and FGF2 and clinical improvement of asthma in children after receiving zinc supplementation.

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Jurnal Respirasi, p-ISSN: 2407-0831; e-ISSN: 2621-8372.
Available at https://e-journal.unair.ac.id/JR. DOI: 10.20473/jr.v6-I.3.2020.61-66

METHODS

The design of this study was a quasi-experimental, pretest-posttest control group, randomized Controlled trial study. This study was conducted on children with mild-severe persistent asthma aged 6-15 years old in Pediatric Respirology Outpatient ClinicDr. Soetomo General Hospital, Surabaya. The study was conducted from January to July 2016. This study was approved by Medical Ethics Committee of Dr. Soetomo Hospital Surabaya. The inclusion criteria were children aged 6-15 years old who met the criteria for mild-severe persistent asthma based on National Pediatric Asthma Guideline 2015, able to perform pulmonary physiological tests, not undergoing immunotherapy, willing to participate in the study, and willing to sign the informed consent. Exclusion criteria include having comorbidities such as pneumonia, tuberculosis, diarrhea, chronic liver or kidney disease, consuming diuretic therapy, malabsorption, and malnutrition. The minimal size of the sample was 11 for each study group. Each patient in the intervention group received zinc syrup supplementation containing zinc sulfate (ZnSO4) 64.9 mg, equivalent to 20 mg elemental zinc every day for 28 days, whereas the control group received placebo. Drugs that have similarities in taste and shape were randomly administered by the pharmacy officer. All patients must be treated with a combination of Inhaled Corticosteroid (ICS) and Long Acting Beta2 Agonis (LABA) as a controller therapy. Blood specimens were collected from all subjects in day 1 and day 28 to determine zinc, EGF, and FGF2 levels by using ELISA method. Clinical improvement was evaluated with C-ACT. All numerical data were analyzed by a descriptive statistical method and the distribution of the sample was analyzed by Kolmogorov-Smirnov test, with a significant value of $p < 0.05$. Independent T-test and Mann-Whitney tests were used to analyze the parametric and non-parametric data. Pre and post-intervention were analyzed by paired T-test. The descriptive data were analyzed by Chi-square test. The results with p values < 0.05 were considered to be statistically significant. A correlation test was conducted to see the correlation between each variable.

RESULTS

A total of 34 patients came and were diagnosed as persistent asthma, 26 patients fulfilled the inclusion criteria, 2 of them lost to follow-up during 2nd week, and 1 patient loss to follow up during the 4th week. A total of 23 patients with complete observation were divided into two groups: intervention group (n = 11) received zinc supplementation 20mg/day and control group (n = 12) received a placebo. They were distinguished by age, sex, weight, height, nutritional status, and asthma classification according to PNAA 2015. The age, sex, zinc levels, body weight, height, andnutritional status in both groups were not significantly different. The characteristics of the sample are presentedin Table 1.

Table 1. Sample characteristics

Subject Characteristics	Treatment Group	Control Group	*P*
Zn level (μmol/l)	5.35(SD 2.34)	4.06(SD 1.68)	0.138
Age (years)	7.81(SD 2.86)	9.35 (SD 2.72)	0.803
Gender			
Male	6	5	0.537
Female	5	7	
Nutritional status			
Normal	8	8	0.752
Malnutrition	3	4	
Weight (kg)	24.5 (SD 7.46)	28.2 (SD 11.98)	0.383
Height (cm)	122.81 (SD 13.77)	129.39 (SD 6.57)	0.315
Family atopic history			
Parents	4	7	0.292
Relatives/family	7	6	0.510
Duration of asthma			
≤ 1 year	4	5	0.795
≥ 1 year	7	7	
Asthma based on frequency			0.482
Mild Persistent Asthma	3	5	
Moderate Persistent Asthma	7	7	
Severe Persistent Asthma	1	0	
Allergy Skin Prick Test			0.755
Food allergy	2	2	
Pet, Food allergy	1	0	
HDM, Food allergy	2	1	
HDM, Pet, Food allergy	6	9	

At the beginning of the study, the control group had lower EGF levels (15.38 ± 3.04) than the intervention group (18.39 ± 5.59) and the difference was quite significant (p = 0.018). Although in the 4th week the mean of EGF levels in the intervention group was significantly different from the control group, it cannot be said that zinc affects in increasing the EGF level, the proportion between pre and post difference must be evaluated. The mean increase of EGF levels after finishing treatment in the intervention group was 55.59 ± 6.48, which was much higher than the control group with a mean of 5.35 ± 5.55. Therefore, there was a significant difference between intervention and control group (p = 0.000) (Table 2).

Table 2. Effect of zinc on EGF levels

	Treatment Group	Control Group	*P*
Pre	18.39 (SD 5.59)	15.38 (SD 3.04)	0.018
Post	73.98 (SD 2.55)	20.73 (SD 4.41)	0.000
P	0.000	0.007	
Delta	55.59 (SD 6.48)	5.35 (SD 5.55)	0.000

The proportion between pre and post differencein FGF2 level after completing the treatment obtained a mean (6.37 ± 1.41) in the intervention group, which was much higher than the mean of the control group (0.72 ± 0.48). A significant difference was obtained between treatment and control group (p = 0.000) (Table 3).

Table 3. Effect of zinc on FGF2 levels

	Treatment group	Control group	*P*
Pre	2.13 (SD 0.35)	2.21 (SD 0.36)	0.568
Post	8.50 (SD 1.52)	2.93 (SD 0.33)	0.000
P	0.000	0.000	
Delta	6.37 (SD 1.41)	0.72 (SD 0.48)	0.000

In this study, subjects were not divided based on severity of asthma. Zinc supplementation was given to all subjects regardless their asthma severity because all subjects have deficiency zinc (Table 4). After supplementation, most of the subjects in both groupshave normal zinc level, there were only 2 subjects in control group who have severe zinc defiency.

Table 4. Initial level of zinc deficiency before supplementation

		Group		Total
		Treatment	Control	
Iniitial Level of Zinc	Moderate Deficiency	3 (27,3%)	1 (8,3%)	4
	Severe Deficiency	8 (72,7%)	11 (91,7)	19
Total		11	12	23

Although there was an increase of the C-ACT score in each group at the end of the study, the mean of the C-ACT score of the treatment group and the control group were significantly different (p = 0.000). The increase in the difference of the C-ACT score in the treatment group (10.27 ± 2.00) was significantly higher than the C-ACT score in the control group (4.50 ± 2.90) (Table 5).

Table 5 Effect of zinc on C-ACT score

	Treatment group	Control group	*P*
Pre C-ACT score	13,00 (SD 9,20)	14,92 (SD 2,46)	0,153
Post C-ACT score	23,27 (SD 2,88)	19,92 (SD 2,71)	0,000
P value	0,003	0,004	
Delta C-ACT score	10,27 (SD 2,00)	4,50 (SD 2,90)	0,000

If we look at the relationship between variables, the increase in EGF and FGF2 levels would be followed by the increase in the C-ACT score. This was shown by the indirectional relationship between ACT score, moderate strength for EGF (r = 0.592; p = 0.003), and strong for FGF2 (r = 0.607; p = 0.002).

DISCUSSION

The ideal measurement method of zinc levels for respiratory tract disease is by the measurement of apical labile zinc concentration in tracheobronchial epithelial cells, but this measurement requires a relatively invasive technique.[6] The measurement of zinc level in this study was using blood plasma samples as the most reliable method, because the tracheobronchial specimen was impossible to be taken, despite the factors that can influence the result.[3]

According to the classification of The Second National Health and Nutrition Examination Survey II of United States (NHANES II) (1976-1980), all the subjects, both intervention and control group, were in a zinc deficiency state, with mean zinc level less than70µg / dl and according to WHO 2001 with zinc levels < 9.18 µmol / l (<60 µg / dl). This is consistent with most of the previous studies that stated zinc levels were lower in asthma patients even though the limits used weredifferent in each study.[2,7–10]

In this study, zinc supplementation was given regardless of the zinc status, because clinically there was no pathognomonic manifestation of zinc deficiency. There was no conflict of ethics about zinc supplementation in this study. This study also excluded the subjects who were at risk for zinc deficiency, such as

patients with malnutrition, diarrhea, etc. Some studies provided zinc supplementation without checking the baseline zinc level, but some researchers gave zinc supplementation only for zinc deficiency patients.[11]

In asthmatic patients with pollutants trigger, oxidative stress will stimulate the epithelium to produce EGF, which is needed for epithelial repair. Studies on children who suffer from asthma attacks found thatthere was an increase of sputum EGF levels that was significantly higher than stable asthma patients and healthy controls. This sputum EGF level examination was measured by ELISA technique. The results of this study support the role of EGF in epithelial regeneration in asthma patients.[12]

Our study showed that zinc supplementation had significantly increased the EGF level of the intervention group compared to those who received placebo. Stimulation or injury causes epithelial damage, which occurs due to an increase in the apoptosis process. This apoptosis process will increase zinc deficiency. All the subjects in this study had zinc deficiency that indicated the epithelial damage process. This epithelial damage will stimulate the increase of EGF production by the respiratory epithelium. With zinc supplementation, EGF activity will increase because the formation of EGF is influenced by matrix metalloproteinase (MMPs). MMPs is a cytokine and growth factor released by epithelial and mesenchymal cells (fibroblasts, inflammatory cells, and chondrocytes). Matrix metalloproteinase is a zinc-dependent endopeptidase group of enzymes. The addition of zinc will increase its activity. This theory explains the role of zinc supplementation in the increase of EGF levels.

The basic fibroblast growth factor, commonly known as fibroblast growth factor-2 (FGF2), is a member of the FGF family, which includes several isoforms that are characterized by their N-termination extension, subcellular distribution, and function. FGF2 is a phenotypic growth factor that induces proliferation of vascular endothelial and smooth muscle cells during angiogenesis, triggers fibroblast migration and proliferation, reverses myofibroblast phenotype, and promotes migration, proliferation, and regeneration during wound healing.[13] A study of FGF2 conducted by Reddington, *et al.*, which took specimens of bronchoalveolar lavage from asthmatic and non-asthmatic patients, found that FGF2 was increased in patients with atopic asthma compared to healthy controls and FGF2 increased in response to allergen exposure indicating FGF2 was involved in the process of airway remodeling in asthma.[14]

In our study, the supplementation of zinc increased the level of FGF2 in the blood. Zinc plays a very important role in the formation of fibroblasts, which maintains the integrity of the basement membraneof the respiratory tract by producing collagen and other extracellular proteins. Therefore, zinc also plays a role in repairing cell damage and remodeling the respiratory tract. In zinc deficiency conditions, fibroblast proliferation and collagen synthesis are disrupted, so that wound healing is delayed. There is a synergistic interaction between growth factor and zinc in the re-epithelialization process and collagen accumulation in the thick layer of injured skin.[15] In this study, zinc supplementation was not related to the remodeling process, but we had to pay attention to the role of FGF2 in the remodeling process.

A study conducted by Lee, *et al*. showed different results about the role of FGF as a protective factor to prevent emphysema in asthma. Lee's study used a sample from non-atopic asthma patients, because the damage caused by fibroblast accumulation in non-atopic asthma patients is irreversible, in contrast to atopic asthma patients who are reversible.[13]

The changes in the C-ACT score in this study showed the improvement of clinical manifestation of asthma by the increase of the score. The intervention group showed better improvement with the mean at the end of the evaluation of 23.27 ($p = 0.003$). The increase in scores also occurred in the control group with a mean score at the end of the study of 19.92 ($p = 0.004$). Because the two groups showed an increase in the C- ACT score, we saw the difference between pre and post-treatment C-ACT scores in both groups. The C-ACT score showed a significant difference, meaning that the difference in scores in the intervention group was higher than the control group ($p = 0.000$).

The increase of the intervention group's scorewas higher because of the administration of zinc supplementation. It would reduce the degree of inflammation through the ability of zinc as an antioxidant and epithelial regeneration agent through the EGF and FGF2 pathways. This score improvement was also supported by the administration of inhaled steroids

as a controller drug. The administration of this controller drug was also able to increase the C-ACT score in the control group. Education related to allergy test procedure was performed in both groups by avoiding foods that were proven to trigger asthma symptoms suchas shortness of breath, coughing, and environmental control.

The results showed that the intervention group had to improve clinical symptoms based on C-ACT scores. This study is in line with the results of previous studies conducted by Blitagi (2009) and Ghaffari (2014).Blitagi conducted a study with zinc, omega 3 fatty acids, and vitamin C supplementation for 38 weeks that were divided into 6 phases in children with moderate persistent asthma who were evaluated clinically using Childhood Asthma Control Test which obtained clinical improvement results indicated by an increase in the C-ACT score.[16]

The mean C-ACT score in the intervention group at the beginning of this study was 13.00 and in thecontrol group was 14.92. The mean value of the C-ACT score in the intervention group provided information that the degree of asthma control in the study subjects was poor, as well as in the control group with a mean of 14.92. Blitagi's study showed a mean initial C-ACT score of 16.5 in the intervention group, and a mean C- ACT score of 16.57 in the control group. At the end of Blitagi's study, there was an increase in the two group scores and there were significant differences between the two groups.[16] At the end of the study, they alsofound an increase in the intervention group, namely 23.27, and the mean in the control group score was 19.92. There were significant differences between the two groups.

Ghaffari conducted a study with 50 mg zinc supplementation daily for 2 months in children with mild asthma who received inhaled corticosteroids. This study showed that there was an improvement in clinical symptoms with reduced coughing, wheezing, and shortness of breath. Ghaffari's study did not use the C-ACT score, thus changes in the parameters of cough, wheezing, and shortness of breath were very subjective.[11] The dose given in Ghaffari's study was 50 mg, while in this study the dose was 20 mg.

CONCLUSION

Zinc supplementation will improve the C-ACT score through the function of zinc as epithelial regeneration by increasing EGF and FGF2.

REFERENCES

1. Rahajoe N, Supriyatno B, Setyanto DB. Pedoman Nasional Asma Anak. *Jakarta UKK Pulmonologi PP IDAI* 2004; 1–4.
2. Al- Selevany B. Role of Serum Zinc Level in Patients with Bronchial Asthma. *WORLD J Pharm Pharm Sci*.
3. Arik Yilmaz E, Ozmen S, Bostanci I, et al. Erythrocyte Zinc Levels in Children with Bronchial Asthma. *Pediatr Pulmonol* 2011; 46: 1189–1193.
4. Batra S, Balamayooran G, Sahoo MK. Nuclear Factor-κB: A Key Regulator in Health and Disease of Lungs. *Arch Immunol Ther Exp (Warsz)* 2011; 59: 335–351.
5. Bernal P, Bauer E, Cao R, et al. A Role for Zinc in Regulating Hypoxia-Induced Contractile Events in Pulmonary Endothelium. *Am J Physiol Lung Cell Mol Physiol* 2011; 300: L874-86.
6. Chinard FP. Quantitative Assessment of Epithelial Lining Fluid in the Lung. *Am J Physiol* 1992; 263: L617-8.
7. Al-Assaf N. Determination of Serum Trace Elements Magnesium, Copper, Zinc, and Selenium in Asthmatic Patients by Atomic Absorption Spectrophotometry. *J Al-Nahrain Univ Sci* 2010; 13: 20–25.
8. Al Salhen K. Role of Serum Trace Elements Magnesium, Copper and Zinc, Level in Libyan Patients with Bronchial Asthma. *IOSR J Biotechnol Biochem* 2015; 1: 97–101.
9. Kakarash TA, Al-Rabaty A. Zinc Status in Children with Bronchial Asthma. *Iraqi Acad Sci J* 2012; 11: 698–703.
10. Katsoulis K, Kontakiotis T, Gerou S, et al. Alterations of Erythrocyte Superoxide Dismutase Activity in Patients Suffering from AsthmaAttacks. *Monaldi Arch chest Dis = Arch Monaldi per le Mal del torace* 2010; 73: 99–104.
11. Ghaffari J, Khalilian A, Salehifar E, et al. Effect of Zinc Supplementation in Children with Asthma: A Randomized, Placebo-Controlled Trial in Northern Islamic Republic of Iran. *East Mediterr Heal J = La Rev sante la Mediterr Orient = al-Majallah al-sihhiyah li-sharq al-mutawassit* 2014; 20: 391– 396.
12. Enomoto Y, Orihara K, Takamasu T, et al. Tissue Remodeling Induced by Hypersecreted Epidermal Growth Factor and Amphiregulin in the Airway after an Acute Asthma Attack. *J Allergy Clin Immunol* 2009; 124: 913–917.
13. Lee B-J, Moon H-G, Shin T-S, et al. Protective Effects of Basic Fibroblast Growth Factor in the Development of Emphysema Induced by Interferon-γ. *Exp Mol Med* 2011; 43: 169–178.

14. Redington AE, Roche WR, Madden J, et al. Basic Fibroblast Growth Factor in Asthma: Measurement in Bronchoalveolar Lavage Fluid Basally and Following Allergen Challenge. *J Allergy Clin Immunol* 2001; 107: 384–387.
15. Chesters JK, Boyne R. Nature of the Zn2+ Requirement for DNA Synthesis by 3T3 Cells. *Exp Cell Res* 1991; 192: 631–634.
16. Biltagi M Al, Baset AA, Bassiouny M, et al. Omega-3 Fatty Acids, Vitamin C and Zn Supplementation in Asthmatic Children: A Randomized Self-Controlled Study. *Acta Paediatr* 2009; 98: 737–742.