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Supplementation of Zinc and the Amino Acid Cysteine towards the TNF-α Status, Calcium, and Anthropometry to the Stunting Infants after Being Given High Doses of Vitamin A

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Abstract

The growth of stunting infants can also be monitored through the anthropometric status, like theweight and the height. There are other indicators that can be used as the indicators of the growth of infant stunting; a biomarker of the bone growth, such as TNF-α and calcium. At the time of the matrix maturation, the bone cells of osteoblast forms the calcium ions as inorganic minerals that can increase the bone mass and speed up the addition height of the children. TNF-α is a pro inflammatory mediator, which is indispensable during the bone growth. Giving high doses of vitamin A has not effectively address the problem of the stunting infants so that the additions of other supplements are required, namely zinc and the amino acid cysteine. Cysteine amino acid speed up the hormone receptor transcription in the reaction of zinc finger protein (ZFP), induction of Transforming Growth Factor β (TGF β), and the formation of Cysteine Rich Intestinal Protein (CRIP) that are strongly associated with the presence of zinc.

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Supplementation of zinc and the amino acid cysteine was conducted over three (3) consecutive months to thestunting infants, who have obtained the high doses of vitamin A. Determination of TNF α levels and calcium were performed in pre and post using Eliza Human TNF α Kit and Eliza Human Calcium Kit. Anthropometry, which include weight and height measured every month. Result show that the provision of zinc and the amino acid cysteine to the stunting infants who have acquired high doses of vitamin A can affect the levels of TNF-α and calcium; and can improve the anthropometric status, which includes weight and height. Giving zinc and the amino acid cysteine is an alternative companion to the highdoses of vitamin A to help repairing the bone growth disorders to the infants stunting.

Keywords: Stunting, Zinc; Cysteine Amino Acids; Vitamin A; TNF-α; Calcium; Weight, Height.

1. Introduction

Stunting or short stature is a nutritional problem gaining well attention both nationally and internationally. The problem of short stature to the infants related to the impaired linear growth. One of the causes of the short infant nutritional problem is the disturbance of the bone growth. In supporting the bone growth, highdoses of vitamin A needs other micronutrients [1]. Zinc is one of themicro minerals that have a role in the protein synthesis and the function of the cellular enzymes so that the role of zinc in the bone growth is enormous. Currently, approximately 20% of the infants' population in the world haverisk of zinc deficiency from the daily food. UNICEP, USAID and WHO concluded that zinc supplementation isvery needed in some countries, including Indonesia [2].

Zinc, in addition to a role in the synthesis of Retinol Binding Protein (RBP), it also plays an important role in the bone cell differentiation process to achieve the optimal linear growth to the children in infancy. In the process of differentiation, the bone cells require zinc amino acid cysteine and histidine. Both of these amino acids are known to work in gene regulation. Klug mentioned it as zinc-finger. Zinc-finger shapes require four amino acid residues as ligands, namely two cysteineand two histidine in each zinc molecule [3]. The second bonding pattern of amino acids and zinc are called Zinc-Finger Protein (PZF). PZF is important for the binding of the transcription factors to DNA by binding histidine and cysteine residues in the transcription factors and nuclear hormone receptors. Zinc puts finger-like secondary structure on the protein. This structure can increase the transcription factor on the gene promoter [4].

Another mechanism that can be done by zinc in the bone growth through positive effects on the stimulant hormonal stimulation is the hormone that is necessary for the differentiation, proliferation, and maturation of lymphocytes T.

Lymphocytes T has a role as cells that can induce TNFα, IFNγ, IL-1, IL-6, and IL-11 that play a role in the regulation of the Growth Hormone (GH) to induce Insulin Like Growth Factor 1 (IGF-1). IGF-1 requiresthe Insulin Receptor Substrate 1 (IRS1) on the proliferation of the bone cells or osteoblasts [5]. Basically, the stunting infant growth may also be seen from the growth of the anthropometry, in this case the weight and height, which in fact at the time of the maturation matrix, the bone osteoblasts form the calcium ions as

inorganic minerals, which can increase the bone mass and speed up the addition of the children's height [6].

Cysteine amino acid accelerate the hormone receptor transcription on the reaction of Zinc Finger Protein (ZFP), induction of Transforming Growth Factor β (TGF β), and the formation of Cysteine Rich Intestinal Protein (Crip) that were strongly associated with the presence of zinc [4]. This research study is to assess the linear growth to the stunting infants through the changes in the levels of TNF-α, Calcium, and anthropometry through the provision of zinc and the amino acid cysteine after being given supplementation of highdoses of vitamin A to the infants stunting.

2. Materials and Methods

This research is an experimental research with randomized pretest posttest control group design. Treatment approach uses double blind methods. The sample in this research werethe infants who pass in the screening with short stature criteria based on the index of the body height/age at -2SD to -3SD, not in ill circumstance. The samples were taken randomly. The research sample were in the two (2) groups, the group given Zinc + cysteine amino acid + highdoses of Vitamin A, and the group given high doses of Vitamin A.

Dosage of the supplements given by Zinc is in the form of zinc sulfate syrup. Giving 5 ml of zinc syrup with zinc sulfate content of 27,45 mg is equivalent to 10 mg of zinc. Zinc supplementation is done every day for three consecutive months. Cysteine amino acid is in the powder form, insipidity, and white as flour. The administered dose is 25 mg/day at the time of the administration for three consecutive months. Supplementation of high doses of vitamin A (200.000 SI) follows the government program conducted simultaneously in August 2015.

Data collected include primary and secondary data. Primary data was obtained from the measurement result of TNF α levels using Human Kit TNF αEliza, calcium levels using Human Kit Calcium Eliza, and anthropometry includes weight and height. Statistical analysis is used to test the hypothesis using a paired test to test the differences in increased levels of TNF-α, calcium, and anthropometric before and after the treatment in each group.

3. Results

The total number of the samples in this research were as many as 30 samples divided into group 1 (one); the treatment in giving Zinc + Cysteine Amino Acids + High Doses of Vitamin A, and group 2 (two) as a control; the infants who have gained a high dosesof vitamin A. The time in doing the research was conducted in August 2015, following the schedule of the government in terms of giving the supplementation of highdoses of vitamin A to the infants simultaneously held throughout Indonesia.

Table 1 shows the nutritional status based on the height/age with a very short category reached 53,34% and short as amounted to 46,66%. If it is seen from the indicators of weight/age of the samples in this research, the samples have good nutritional status as many as 93,34% in the first group and 73,33% of in the second group.

Table 1: The Characteristic Sample of the Stunting Infants

Characteristic Sample	Group 1		Group 2	
	n	%	n	%
Age (month)				
≤ 30	6	40	4	26.67
>31	9	60	11	73.33
Total	15	100.00	15	100.00
Sex				
Male	6	40	5	33.34
Female	9	60	10	66.66
Total	15	100.00	15	100.00
Nutritional Status				
Height/Age				
Short	7	46.66	7	46.66
Very Short	8	53.34	8	53.34
Total	15	100.00	15	100.00
Weight/Age				
Good Nutrition	14	93.34	11	73.33
Malnutrition	1	6.66	4	26.67
Total	15	100	15	100

Table 2: Differences of TNF α Level between Before and After the Treatment to the Stunting Infants Sample

Group 1	Mean	Standar Deviasi	P value
Before	23,320	2,1078	0,008
After	26,066	4,2525	
Group 2			
Before	22,286	1,9522	0,259
After	23,253	2,1563	

Table 2 illustrates that the average level of TNF-α before giving zinc + Cysteine & + Vitamin A (group 1) is 23.320 pg/ml with a variation of 2.1078 pg/ml. After the administration, there is an increase of 2.746 pg/ml to an average of 26.066 pg/ml with a variation of 4.252 pg/ml. Results of the statistical test (t test) generate P value=0,008. It means that there are significant differences in the levels of TNF α between before and after the administration of zinc + cysteine + vitamin A.

Table 3: Differences of Calcium Levels between Before and After the Treatment to the Stunting Infants Sample

Group 1	Mean	Standar Deviasi	P value
Before	8,5533	1,5468	0,0001
After	17,4067	3,4658	
Group 2			
Before	14,0800	5,9538	0383
After	16,6133	7,1607	

Calcium levels of the measurement results is shown in Table 3, which suggests that the average calcium levels before giving zinc + Cysteine & + Vitamin A (group 1) is 8,5533 mg/ml with a variation of 1,5468 mg/ml. After the administration happens, there is an increase of 8,8534 mg/ml to an average of 17,4067 mg/ml with a variation of 3,4658 mg/ml. Based on the results of the statistical tests (t test), P value=0,0001 is obtained. This means that there are significant differences in the calcium levels between before and after giving zinc + Cysteine + Vitamin A.

Table 4: Differences of Weight between Before and After the Treatment to the Stunting Infants Sample

Group 1	Mean	Standar Deviasi	P value
Before	12,81	1,71	0,0001
After	14,70	1,44	
Group 2			
Before	11,95	1,39	0,0001
After	12,71	1,37	

In table 4, it appears that there is an increase of 1,89 Kg weight in group 1, and 0,76 kg in group 2. Statistical test results showed the P value = 0.0001. This means there is a significant change in Weight both group 1 and group 2.

Height measurement results can be explained in Table 5.Compared to weight, the conclusion can be drawn that the weight and height equally increased.

Table 5: Differences of Height between Before and After the Treatment to the Stunting Infants Sample

Group 1	Mean	Standar Deviasi	P value
Before	85,06	3,57	0,0001
After	86,67	3,50	
Group 2			
Before	83,18	3,53	0,0001
After	84,71	3,43	

In Table 5, it appears that there is an increase of 1.61 cm height in group 1, and 1.53 cm in group 2. Statistical test results show the P value =0.0001. It means that there is a significant change in height either in group 1 or in group 2.

4. Discussion

Bone growth disorders can cause the disruption of the linear growth, so it causes the children under five to suffer the short nutritional status [7]. One treatment done to address this problem is by giving the supplementation of the highdoses of Vitamin A. Vitamin A effectsto the protein synthesis in the cell growth, including the bone cells [7, 8].

This research provides the additional zinc and the amino acid cysteine in the supplementation of Vitamin A, which is already implemented as a program of the Indonesian government. Zinc is potential as an immune stimulant on the immune-cellular response. The effect of zinc on the immune system, among others, is an inhibitor of apoptosis that is very necessary when the bone growth through the suppression of the glucocorticoid hormone secretion, which is an apoptosis inducer for the timosit and precursor T cells [9]. Mature T Lymphocytes induces the receptors of the expression of TNFα, IFNγ, IL-1, IL-6, and IL-11. These cytokines are involved in the process of the bone remodeling [7, 10].

Results of the analysis of TNF α level in this research show the P value of 0,008 in the sample given zinc + cysteine + Vitamin A. This shows that there is an impact from the treatment of TNF α level to the stunting infants. The main goal of treatment procedure of this research is to look at the status of the bone repair. The connection of TNF-α in repairing the bone growth plays a role in stimulating the osteoclasts to stimulate the production of RANKL by the stromal cells, and also induces the secretion of RANKL by T lymphocytes, B lymphocytes, and endothelial cells to induce the osteoclast formation indirectly. TNF-α also stimulates the production of M-CSF by the stromal cells. Osteoclast differentiation factor (ODF, also called RANKL/TRANCE/OPGL) stimulates the osteoclast progenitors on the monocytes/macrophages into the osteoclasts in the presence of the macrophage colony-stimulating factor (M-CSF) [11].

The process of bone remodeling is a process that generates the growth and the bone turnover. Remodeling process sequence is a sequence that is certain, namely activation resorption and formation (ARF). The phase

between the ARF has a between phase, which is reversal phase involving some mononuclear cells such as macrophages that form the cemen line to limit the process of the resorption and glue the old and the new bones [12].

Another local factorsthat play a role in the remodeling process are IGF, TGF, Fibroblast-Growth Factor (TGF), Platelet Derived Growth Factor (PDGF), Interleukin (IL), Tumor Necrosis Factor α (TNF α) Colony Stimulating Factor (CSF), and interferon (IFN) [3. 13].

The role of cytokines in the early remodeling is very important. Cytokines are the protein mediator and the mediator in the physiological pathology response to the infection. TNF α, besides playing a role in the inflammatory process in the bone remodeling, is also a growth factor because there are in almost all changedcells, including osteoblasts [9]. TNF α, also called as cachectic, is an immune cytokine produced by the macrophages. TNF-α can induce the expression of an autocrine growth factor, increase the cellular response to the growth factors, and induce the signaling pathways that lead to the proliferation. TNF-α acts synergistically with EGF and PDGF on some cell types, including the bone cells [11].

TNF-α may increase the osteoclast genesis through two different mechanisms, namely TNF-α first affects the osteoclast genesis at the osteoclasts precursors in the bone marrow by the basic cells to the air differentiation into c-Fms+/CD11b+/RANK+/- of the osteoclasts progenitors through the independent mechanisms of RANKL/RANK. Osteoclast precursors are entered into the blood vessels and peripheral tissues, and thenthey differentiate into the mature osteoclasts (dependent mechanism) whose role is to speed up the process of the bone resorption. Another indicators of the bone repair analyzed in this research are the levels of the calcium. The results show the P value=0,0001. It indicates that this research improves the treatment of the plasma calcium status, where nearly 50% of the calcium is calcium ionized plasma with the reference to 1,0-1,2 mmol/l. The rests are not bound ionized to the citrate. The other 50% of plasma calcium is bound to the proteins, primarily albumin. Physiologically, the calcium is active free and responsible to the bone growth and the neuromuscular effects [4,7,14].

Zinc interacts with the hormones involved in the bone growth, such as somatomedin C, osteocalsin, testosterone, thyroid hormone, and insulin. Therefore, zinc is closely associated with the bone metabolism. Zinc acts positively to the growth, including the bone growth [15]. Long bone growth disorders, in addition to the elements influenced by the nutrients, are also affected by the disruption of the hormone regulation during the formation of the long bones. One hormone disturbed is IGF-1. IGF-1 plays an important role as an introduction to the signal when the elongation of the bone cells happens. The existence of IGF-1 is influenced by the levels of zinc. Proven zinc supplementation can correct the linear growth [5].

This research results a real change in the anthropometric, ieweight and height in both groups. This shows that zinc, amino acids cysteine, and vitamin A are as good as for the anthropometric status. Zinc can change the appetite controls by the direct way on the central nerve system and change the levels of the receptor responsiveness to the neurotransmitter. Zinc deficiency is usually followed by the changes in ability and acuity of taste and smell through the disruption of the appetite, and eventually can lead to low nutrient intake and

weight loss as well as the disruption of the linear growth [8,11].

The role of the amino acid cysteine in the process of transcription is one of the cell differentiation processes, where the process is initiated by the binding of the promoter and transcription cofactor that cause the transcription of DNA. For instance, morphogenetic bone protein causes mRNA transcription involved in osteogenesis [16,17]. By nature, cysteine has a special role in the structure of the protein, such as insulin, IGF, growth factors, immunoglobulin, or antibodies [18]. The area of cysteine between the fifth and the sixth is displayed on the surface of the molecule and receptor binding as seen in the beginning of the transcription of the cell. Cysteine knot is a monomer form of the growth factor [17, 19].

5. Conclusion

Giving zinc and the amino acid cysteine to the stunting infants who have acquired high doses of Vitamin A can affect the levels of TNF-α and calcium levels; and can improve the anthropometric status, which includes weight and height.

Giving zinc and the amino acid cysteine is acompanionalternative to the highdoses ofVitamin A to help repairing the bone growth disorders to the stunting infants.

References

[1] Coly AN. Preschool Stunting , Adolescent Migration , Catch-Up Growth , and Adult ... 2006;

[2] Imdad A, Bhutta ZA. Effect of preventive zinc supplementation on linear growth in children under 5 years of age in developing countries : a meta-analysis of studies for input to the lives saved tool. BMC Public Health [Internet]. BioMed Central Ltd; 2011;11(Suppl 3):S22. Available from: http://www.biomedcentral.com/1471-2458/11/S3/S22

[3] Shapiro F, Nir I, Shinder D, Silanikove N. Stunting syndrome in broilers: effect of electrolytes in drinking water on performance and intestinal glucose transport. Br Poult Sci. 1999;40(4):501–10.

[4] Krishna SS. Structural classification of zinc fingers: SURVEY AND SUMMARY. Nucleic Acids Res [Internet]. 2003;31(2):532–50. Available from: http://nar.oxfordjournals.org/lookup/doi/10.1093/nar/gkg161

[5] Guntur AR, Rosen CJ. IGF-1 regulation of key signaling pathways in bone. Bonekey Rep [Internet]. Nature Publishing Group; 2013;2(October):1–6. Available from: http://dx.doi.org/10.1038/bonekey.2013.171

[6] Park KH, Park B, Yoon DS, Kwon S, Shin DM, Lee JW, et al. Zinc inhibits osteoclast differentiation by suppression of Ca 2 + -Calcineurin-NFATc1 signaling pathway. Cell Commun Signal [Internet]. Cell Communication and Signaling; 2013;11(1):1. Available from: Cell Communication and Signaling

[7] Of J, Pediatrics T. Zinc deficiency : A contributing factor of short stature in growth hormone deficient children Zinc Deficiency : a Contributing Factor of Short Stature in Growth Hormone Deficient Children.

2003;(November 2015):4–6.

[8] Santos MA. Hipovitaminose A em escolares da zona rural area in Minas Gerais , Brazil. 2005;18(3):331–9.

[9]. Hönscheid A, Rink L, Haase H. T-Lymphocytes : A Target for Stimulatory and Inhibitory Effects of Zinc Ions. 2009;132–44.

[10]. Sitokin K, Proses P, Jaringan D. tumor necrosis factor. :9–28.

[11] Reurings M, Vossenaar M, Doak CM, Solomons NW. Stunting rates in infants and toddlers born in metropolitan Quetzaltenango, Guatemala. Nutrition [Internet]. Elsevier Inc.; 2013;29(4):655–60. Available from: http://dx.doi.org/10.1016/j.nut.2012.12.012

[12] Surkan PJ, Siegel EH, Patel S, Katz J, Khatry SK, Stoltzfus RJ, et al. scores of infants and toddlers. 2014;29(3):542–8.

[13] Biokimia T. Mekanisme Seluler dan Molekular Resistensi Insulin. 2012;

[14]. Veterinary B, Journal M. Evaluation of Zinc Oxide Nanoparticles for Insulin , Insulin Receptors and Insulin Receptors Substrates Gene Expression in Streptozotocin-Induced Diabetic Rats. 2010;166–74.

[15] Alkaladi A. Effect Of Zinc Deficiency And Supplementation On Insulin Signaling In Chickens. 2013;7(1):104–8.

[16]. Witarto AB. Protein Engineering : Perannya dalam Bioindustri dan Prospeknya di Indonesia (The Role of Protein Engineering in Bioindustry and Its Prospect in Indonesia). Bioteknologi [Internet]. 2001;(1):1–7. Available from: http://www.jaist.ac.jp/~witarto/witarto_zoa.pdf

[17] Grudkowska M, Zagdańska B. Multifunctional role of plant cysteine proteinases. Acta Biochim Pol. 2004;51(3):609–24.

[18] Piste P. Cysteine-master antioxidant. Int J Pharm , Chem Biol Sci. 2013;3(1):143–9.

[19] Marino SM, Gladyshev VN. Analysis and functional prediction of reactive cysteine residues. J Biol Chem. 2012;287(7):4419–25.