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Original Article

The relationship between umbilical cord zinc and allergic symptoms, and its negative correlation with toddler zinc: a prospective study

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ABSTRACT

The normal balance of the immune response begins early in life. Previous studies have proven the role of zinc in strengthening the inhibition of Th2 by Th1. Treg plays a role in maintaining balance of Th1 and Th2. The main purpose of this study was to determine the factors associated with allergic symptoms in toddlers, including umbilical cord zinc, infant IgE and toddler zinc. Another objective was to determine the differences in the expression of Foxp3 and zinc at 3 years of age between groups based on medical history since birth. This study was a prospective study involving 53 participants. Subjects were observed from birth to the age of 3 years. Blood zinc levels were measured at birth and at 3 years of age. Total IgE was measured at 4 months of age and Foxp3 expression was measured at 3 years of age. The children medical history was taken from medical records, questionnaires and home visits. There was a correlation of low umbilical cord zinc levels <65 µg/dL with allergy symptoms ($p = 0.034$). There were no differences in Foxp3 expression and toddler zinc levels between groups according to health history factors from birth to 3 years of age ($p>0.05$). There was a significant negative correlation between umbilical cord zinc levels and blood zinc at 3 years of age ($r = - 0.354$, $p = 0.009$). In addition, the results show that there was a positive correlation between IgE levels and umbilical cord blood zinc levels ($r = 0.282$, $p = 0.04$). In conclusion, low umbilical cord zinc levels were associated with allergic symptoms in toddlers. There was no association between medical history factors in the first 3 years of life with Foxp3 Treg expression and zinc levels in toddlers. Higher levels of umbilical cord zinc tend to be followed by lower levels of zinc at 3 years of age.

KEYWORDS: umbilical cord zinc, toddler zinc, toddler allergy symptoms, Foxp3, IgE.

INTRODUCTION

The prevalence of allergic and autoimmune diseases tends to increase. Therefore, background research related to Th1/2/17/Treg cell differentiation dysregulation is still needed. Exposure to the environment early in life can have a major effect on the individual's immune cell differentiation patterns [1].

Differentiation and proliferation of naive CD4+ into Th1, Th2, Th17 and Tregs are influenced by genetic and epigenetic factors. Epigenetic factors are closely related to the history of daily environmental exposure. The exposure factors included a history of exclusive breastfeeding, complementary feeding, formula milk, a history of allergies, repeated infections, and exposure to cigarette smoke. The transcription factors of Th0 cells have high

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flexibility and plasticity at a young age. Therefore, epigenetic factors can have a major influence on their differentiation into T-bet (Th1), Gata3 (Th2), Foxp3 (T regulatory), RORγt (Th17), and Bcl6 (Tfh).) Epigenetic factors can alter the phenotype of helper T cells. Furthermore, it can change gene expression and the pattern of cytokines produced [1, 2]. The proliferation and differentiation of T cells is initiated intrauterine. The plasticity of naive T cells is higher during early life than in older people and will continue to decline in the elderly. This has been demonstrated by an *in vitro* study that found the presence of memory T cells (CD45RA-), naive T cells (CD45RA+), and CD25Treg in umbilical cord blood. In this case the CD25Treg population was quite prominent. On the other hand, there was no CD25+Treg in the blood of the elderly. The thymus in infants develops rapidly, and this function declines with the aging process of humans [3].

An *in vitro* study in Sweden reported the effect of breast milk and exposure to mitogenous lipopolysaccharide on peripheral mononuclear cell cultures derived from infant umbilical cord blood. Breast milk was collected from Swedish mothers, immigrant mothers (not Swedish) and mothers living in Mali. The result showed that the group exposed to breast milk from immigrant mothers living in Sweden had lower levels of proinflammatory cytokines (IL)-6 and CXCL-8/IL-8 than the group exposed to breast milk from mothers living in Mali and native Swedish mothers. This research proved that the effect of breast milk was influenced by race, country and place of residence. In addition, breast milk has a direct effect on inflammatory cytokine patterns in early life [4]. Previous *in vitro* studies have shown that zinc supplementation reduces the proliferation of naive Th cells into Th2 and Th17 subtypes including their cytokines. In conclusion, zinc plays a significant role in the pathogenesis of allergic and autoimmune diseases [5].

It has been shown that zinc affects IFN-γ levels but does not affect IL-6, IL-4 or IL-10 levels. It has been proven that zinc affects IFN-γ levels but does not affect IL-6, IL-4 or IL-10 levels. IFN-γ enhances differentiation to Th1 cells, whereas IL-10 inhibits Th1. Zinc can inhibit Th2 through amplification of Th1 [6]. Zinc deficiency triggers stress on macrophages resulting in the release of inflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-γ [7].

Research on the role of zinc in immune responses has been carried out *in vitro* and in animals, but further prospective research is needed related to the natural immune response of humans from birth to later age. The focus of our research was to identify factors that could influence the incidence of allergies at a young age. The factors that we studied included neonatal zinc, infant IgE, type of delivery, family history of allergies, maternal allergic atopy, environmental exposure (pets, cigarette smoke), history of food consumption by pregnant/ breastfeeding women, non-exclusive breastfeeding, early formula feeding (cow's milk allergen), early complementary foods (food allergens), health status including a history of infection and infant allergies. Another objective was to determine differences in Foxp3 expression and zinc levels of toddlers (aged 0-3 years) between groups based on health history.

METHODS

This prospective study was conducted on infants born at Sultan Agung General Hospital and Bangetayu Health Center Semarang City from September to November 2017. Inclusion criteria were healthy newborns, term pregnancy, birth weight ≥ 2500 g, and healthy maternal conditions. This cohort study initially involved 88 infants, but ultimately only 56 subjects whose parents were still willing to participate in the study until their children reached the age of 3 years. Three samples were not eligible for blood analysis, and thus 53 toddlers participated in this study.

This research was assisted by midwives (health surveillance officers) who made home visits. Research data were obtained from questionnaires by interview and by telephone. Other data were obtained from the medical records of Sultan Agung General Hospital, Bangetayu Health Center and Genuk Health Center.

Measurements of zinc and Foxp3 levels were carried out in the GAKI laboratory, Faculty of Medicine, Diponegoro University, Semarang. Ethical clearance was obtained from the Commission of Bioethics of Medical Research/Health, Faculty of Medicine,

Sultan Agung Islamic University Semarang. This study was conducted after receiving an ethical license and parental consent. Statistical software used was SPSS 21. Hypothesis testing used includes unpaired t test, Pearson correlation, Chi-square test, Fisher's exact test and logistic regression.

Clinical analysis

Children who have experienced symptoms of bronchial asthma, allergic rhinitis, and atopic dermatitis in the previous year were characterized as toddlers with allergic symptoms. In addition the children had sleep disturbances at night due to coughing or itching or runny nose. To detect allergy symptoms, a modified questionnaire was used based on the ISAAC criteria, the Hanifin-Rajka criteria, and an allergy early detection card according to the Allergy Immunology UKK, Indonesian Pediatrician Association [8, 9].

Symptoms of bronchial asthma include recurrent chronic cough with attacks >2 weeks or recurrence >2 times in 3 consecutive months. Other symptoms were cough with wheezing and shortness of breath. These symptoms reduced after being given bronchodilator inhalation therapy at the nearest health service. It was considered as a symptom of asthma if the diagnosis of asthma was carried out by a pediatrician. It was considered allergic rhinitis if the toddler had symptoms of frequent sneezing or runny nose triggered by cold air or a lot of dust, and a history of frequent nasal congestion. These symptoms are experienced for more than a month or almost every month and without fever or sore throat. Toddlers suffered from atopic dermatitis if they experienced symptoms of itching on the skin accompanied by redness for > 6 months (there were periods of remission and relapse). Skin lesions could be urticaria or eczema with a predilection around the neck, folds of the elbows/elbows, folds of the knees/knees, or under the buttocks. Toddlers also had a history of dry skin.

The risk factors that might affect the incidence of allergies in children in this study were early formula feeding (< 1 month of age), early complementary feeding before 4 months of age, non-exclusive breastfeeding, exposure to pets (dogs, cats, birds), exposure to cigarette smoke, history of frequent acute respiratory infections (ARI) in the first 4 months of age (≥ 2x), history of atopic allergies before 4 months of age (confirmed by positive Skin Prick test), and having many siblings (> 3 siblings).

Zinc level analysis

The first measurement of blood zinc levels was done in umbilical cord blood (at birth) and the second was done in peripheral blood at the age of 3 years. Zinc analysis was performed using the Atomic Absorption Flame Emission Spectrophotometer (AA-6401F Shimadzu, Japan). Toddler blood samples were collected at relatively the same time between 8 AM to 12 AM. Zinc levels were considered low if the levels were below 65 g/dL[10].

Analysis of IgE levels

Total IgE were measured at 4 months of age using the Chemiluminescent Immunometric Assay (Immulite 2000, Siemens USA) technique. Analysis of IgE levels was done by Prodia laboratory. IgE levels are considered high when > 29IU/mL.

Foxp3 expression analysis

ELISA technique (Elabscience E-EL-H1104) was used to measure Human Forkhead Box Protein P3 (Foxp3) expression.

RESULTS

This research was originally part of a prospective study of 110 pregnant women who agreed to participate in the cohort study, but 28 dropped out for various reasons such as severe illness in newborns, failure of umbilical cord blood extraction, and parents moving out of town. Then there were 88 mothers and their babies, but in the end only 56 parents agreed that their babies be observed until the age of 3 years. Unfortunately 3 toddler blood samples did not meet the requirements; so only 53 toddlers were left. Among the 53 toddlers, 19 toddlers experienced allergy symptoms. In fact two of them were suffering from atopic allergies since the age of 4 months. Of the 19 toddler allergy symptoms, the most observed symptom was atopic dermatitis (53%), followed by rhinitis allergy (26%). 11% had two symptoms, and 10% had bronchial asthma.

Table 1 presents an analysis of the relationship between toddler allergy symptoms and characteristics factors, as well as other factors. Characteristic factors include gender, birth, and number of siblings. Other factors include history of non-exclusive breastfeeding, early formula feeding (before 1 month of age), low umbilical cord zinc levels (<65 ug/dL), a history of atopic allergy in infants, and total infant IgE levels. The results of data analysis shown in Table 1 prove a significant relationship between umbilical cord zinc levels and toddler allergy symptoms, p = 0.034. Meanwhile, other factors shown in Table 1 were not significantly related to toddler allergy symptoms, p > 0.05.

Table 2 shows the relationship between toddler allergy symptoms and several factors (characteristics

Table 1. The association between characteristic factors and other factors from birth with toddler allergy symptoms.

Group	Toddlers with allergy symptoms	Toddlers without allergy symptoms	RR(95%C.I)	p*
Gender				
Male	9	21	0.69(034-1.42)	0.311*
Female	10	13		
Type of delivery				
Spontaneous	5	6	1.36(0.63-2.96)	0.456*
Caesarean section	14	28		
Maternal atopic allergy history				
Yes	4	7		
No	15	27	1.02(0.42-2.46)	0.968*
Family allergy risk factors				
High- moderate	9	16	1.01(0.49-2.07)	0.983*
low	10	18		
Number of children ≥ 3				
Yes	6	8	1.29(0.61-2.72)	0.524*
No	13	26		
No siblings				
Yes	4	12	0.62(0.24-1.57)	0.279*
No	15	22		
Non-exclusive breastfeeding				
Yes	10	13	1.45(0.71-2.97)	0.311*
No	9	21		
Early infant formula feeding				
Yes	4	11	0.68(0.27-1.71)	0.381*
No	15	23		
History of infants allergies				
Yes	2	5	0.77(0.23-2.65)	0,509**
No	17	29		
High infant IgE levels				
Yes	4	3	1.75(0.82-3.76)	0.234**
No	15	31		
Low umbilical cord zinc levels				
Yes	6	3	2.26(1.18-4.32)	0.034*
No	13	31		

*Chi square test **Fisher test

Table 2. The relationship between toddler allergy symptoms and several exposure factors including history of illness.

Group	Toddlers with allergy symptoms	Toddlers without allergy symptoms	RR(95%C.I)	p*
Food consumption habits during pregnancy				
Frequently eating eggs	15	21	1.77(0.69-4.53)	0.199*
Rarely eat eggs	4	13		
Frequently drinking cow's milk	12	22	0.96(0.46-2.02)	0.910*
Rarely drinking cow's milk.	7	12		
Frequently eat shrimp/shellfish	4	4	1.50(0.67-3.36)	0.365*
Rarely eat shrimp/shellfish	15	30		
Food consumption habits during breastfeeding				
Frequently eating eggs	12	24	0.81(0.39-1.68)	0.578*
Rarely eat eggs	7	10		
Frequently drinking cow's milk	5	9	0.99(0.44-2.26)	0.990*
Rarely drink cow's milk.	14	25		
Frequently eat shellfish/shrimp	0	2	1.59 (1.3-1.9)	0.407**
Rarely eat shrimp/shellfish	19	32		
Frequent acute respiratory infections in the first 4 months of life				
Yes	8	11	1.30(0.64-2.67	0.478*
No	11	23		
House pet				
Yes	10	24	0.62(0.31-1.26)	0.191*
No	9	10		
Toddlers' history of frequent infection				
Yes	9	17	0.94(0.45-1.92)	0.854*
No	10	17		
Exposure to cigarette smokes for the past 1 year				
Yes	7	16	0.76(0.36-1.62)	0.472*
No	12	18		
Low zinc levels in toddlers				
Yes	3	2	1.80(0.79-4.09)	0.336**
No	16	32		

*Chi square test **Fisher test

and other exposure factors). These exposure factors include foods that were often consumed by pregnant/breastfeeding mothers, infections during infancy, infections in the past year, exposure to cigarette smoke as a baby, exposure to cigarette smoke in the past year, pets and zinc levels at 3 years of age. From Table 2, it can be seen that there is no significant relationship between toddler allergy symptoms and all of these factors, $p > 0.05$.

Table 3 presents a multivariate analysis (logistical regression) of all the factors listed in Table 1 and Table 2 whose p-value <0.25. The purpose of logistic regression analysis was to determine the factors most associated with toddler allergic symptoms. The result showed that toddlers with low umbilical cord zinc levels had 6.22 times more risk of allergy symptoms than controls ($p = 0.023$).

Table 3. Risk factors for toddler allergy symptoms.

Risk Factors	Toddlers with allergy symptoms	Toddlers without allergy symptoms	p*	OR(95%C.I)
House Pet				
Yes	10	24	0.609	0.71(0.19-2.66)
No	9	10		
Exposed to cigarette smoke before the age of 4 months				
Yes	12	27	0.235	0.43(0.11-1.73)
No	7	7		
Frequent consumption of eggs during pregnancy				
Yes	15	21		
No	4	13	0.180	2.64(0.64-10.92)
High infant IgE levels				
Yes	4	3	0.096	4.15(0.78-22.14)
No	15	31		
Low umbilical cord zinc levels				
Yes	6	3	0.023	6.22(1.29-30.09)
No	13	31		

*logistic regression analysis

Table 4 explains that there is no difference in the mean Foxp3 expression between groups based on medical history from birth to 1 year of age.

Table 4 and Table 5 present the differences in the mean Foxp3 expression between groups according to medical history from birth to 3 years of age. The results of statistical tests showed that there was no significant difference in Foxp3 expression between groups according to previous medical history, $p > 0.05$.

Table 6 presents data on the difference in the mean toddler zinc levels between groups based on several factors. These factors include a history of infection during infancy, mode of delivery, socio-economic level, non-exclusive breastfeeding, early formula feeding, history of atopic allergy in infants, and total infant IgE levels. There was no difference in toddler zinc levels between the two groups based on all previous medical history factors, $p > 0.05$.

Table 6 describes the history of infants with high IgE levels having lower toddler zinc levels compared to controls (85.43 ± 19.26 vs 109.39 ± 31.61), although not significant, $p = 0.058$.

Figure 1 proves that there is a significant negative correlation between umbilical cord zinc levels and toddler zinc levels ($r = -0.354$ $p = 0.009$).

The result of Pearson correlation between infants' zinc level and infants zinc IgE, showed that there was a non-significant negative correlation, $r = -0.199$, $p = 0.396$, as well as an insignificant negative correlation between FoxP3 levels and infants' total IgE, $r = -0.124$, $p = 0.377$ (Figure unavailable).

Figure 2 illustrates the evidence of significant positive correlation between umbilical cord zinc level with total IgE level at 4 months of age, $r = 0.282$ $p = 0.040$.

DISCUSSION

The results of our study proved that the allergic symptoms of toddlers were associated with a decrease in umbilical cord zinc levels. Our results are similar to a cross-sectional study in Korea in schoolchildren. This study proved that an increase in total/specific IgE levels correlates with a decrease in zinc levels [11]. It seems that decreased zinc levels are associated with allergic symptoms or elevated IgE levels. However, our study did not

Table 4. Differences in Foxp3 expression levels based on medical history at infancy.

Medical history of infancy	n	Mean(SD) ng/mL**	Mean Comparison (95%C.I)	p*
Maternal atopic allergy history				
Yes	11	0.20 (1.52)	0.68 (0.42 – 1.10)	0.117
No	42	0.30 (2.14)		
Non-exclusive breastfeeding				
Yes	23	0.29 (2.41)	1.12 (0.75 – 1.68)	0.565
No	30	0.26 (1.77)		
Early infant formula feeding				
Yes	15	0.30 (2.54)	1.15 (0.74 – 1.79)	0.527
No	38	0.26 (1.87)		
Very short duration of breastfeeding				
Yes	3	0.45 (2.56)	1.68 (0.71 – 3.94)	0.232
No	50	0.26 (2.02)		
Early weaning food				
Yes	6	0.26 (2.32)	1.08 (0.44 – 1.75)	0.814
No	47	0.28 (2.04)		
Frequent acute respiratory infections in the first 4 months				
Yes	19	0.30 (2.42)	1.15 (0.76 – 1.75)	0.492
No	34	0.26 (1.86)		
House pet				
Yes	34	0.30 (2.24)	1.32 (0.87 – 1.98)	0.186
No	19	0.23 (1.82)		
Exposure to cigarette smoke				
Yes	39	0.25 (1.91)	0.72 (0.46 – 1.12)	0.137
No	14	0.35 (2.39)		
High infant IgE levels				
Yes	7	0.86 (1.65)	0.81 (0.45 – 1.47)	0.485
No	46	0.28 (2.11)		
Low umbilical cord zinc levels				
Yes	9	0.22 (2.03)	0.78 (0.45 – 1.28)	0.295
No	44	0.29 (2.06)		
History of infants allergies				
Yes	7	0.24(0.17)	0.87(0.48-1.56)	0.630
No	46	0.28(0.21)		

*Independent sample t test **nanogram /mL SD = standard deviation C.I = Confident Interval

prove that toddler zinc levels were not associated with allergic symptoms. Research in Nigeria showed that children with asthma had lower zinc levels than controls [12]. Another case-control study also proved that zinc levels were lower in children with asthma [13].

In this study, children aged 3 years with allergic symptoms were not associated with a decrease in toddler zinc levels, unlike previous studies that showed that there was a decrease in zinc levels in children with asthma. This study only covers a small proportion (10%) of subjects with asthma symptoms. It is possible that other types of allergies were not accompanied by a significant decrease in toddler zinc levels. Early life zinc levels play a more important role in the development of allergic symptoms in infancy than toddler zinc, as evidenced in our study.

Table 5. Differences in Foxp3 levels based on past year's medical history.

Medical history of the past year	n	Mean(SD) ng/mL**	Mean Comparison (95%C.I)	p*
Toddlers with allergy symptoms				
Yes	19	0.26 (1.96)	0.91 (0.60 – 1.39)	0.667
No	34	0.28 (2.30)		
Toddlers exposed to cigarette smoke				
Yes	23	0.33 (2.24)	1.38 (0.93 – 2.04)	0.109
No	30	0.24 (1.86)		
History of frequent infections in toddlers				
Yes				
No	26	0.26 (1.93)	0.88 (0.59 – 1.31)	0.507
	27	0.29 (2.18)		
History of frequent acute respiratory infections				
Yes	21	0.27 (2.03)	0.96 (0.64 – 1.44)	0.832
No	32	0.28 (2.09)		
Very rarely experienced infection				
Yes	8	0.41 (2.51)	1.59 (0.93 – 2.75)	0.091
No	45	0.26 (1.95)		
Very rarely experienced acute upper respiratory tract infection				
Yes	12	0.36 (2.35)	1.38 (0.86 – 2.21)	0.175
No	41	0.26 (1.95)		
Low zinc levels in toddlers				
Yes	5	0.23 (1.75)	0.83 (0.42 – 1.64)	0.587
No	48	0.28 (2.09)		

*Independent sample t test **nanogram /mL SD = standard deviation C.I = Confident Interval

Our important finding was the negative correlation of umbilical cord zinc levels with toddler zinc levels. In this case, lower zinc levels in neonates tended to be followed by higher zinc levels after 3 years of age. On the other hand, if the neonates have lower zinc levels, the zinc levels at 3 years of age are lower. In addition, those who had a history of atopic allergy in infancy did not complain of allergy symptoms during the previous year until now. It should be noted that these toddlers' zinc levels were higher than before (umbilical blood zinc levels). The effects of zinc have been demonstrated from our previous study as follows: 1. there is an association of atopic allergic infants with lower cord zinc levels, 2. atopic atopic allergy in infants is not associated with high total IgE levels >29 IU/mL[14]. Studies in Iran concluded that there was no association of zinc supplementation with changes in total IgE levels in asthmatic patients despite clinical improvement. This fact suggests a short-term role of zinc in reducing allergic symptoms [15].

Our study supports that umbilical cord zinc levels were associated with the appearance of allergic symptoms at an older age. It is possible that early life zinc levels play a role in preventing allergic symptoms in individuals with high IgE levels. Zinc plays a role in the regulation of long-term immune responses and influences the differentiation of naive helper T cells [6, 7]. Previous studies have proven the association of Hypozincemia with severe atopic dermatitis and increased IgE [16].

Intracellular zinc metabolism is very dynamic, one of which is the zinc wave mechanism or zinc transporter proteins (ZnT & ZIP), which regulates zinc levels in various parts of the body. Therefore, zinc levels fluctuate according to the influence of the stimulus received by the body, zinc intake from the placenta during the fetus and intake after

Table 6. Differences in toddler zinc levels based on infancy health history.

History before 3 years old	n	Toddlers zinc levels Mean(SD) µg/dL	MD(95%C.I)	P*
Frequent acute respiratory infections in the first 4 months				
Yes	19	100.37(26.17)	-9.13(-27.07-8.81)	0.312
No	34	109.50(33.64)		
High infant IgE levels				
Yes	7	85.43(19,26)	-23.96(-48.74-0.81)	0.058
No	46	109.39(31.61)		
Non-exclusive breastfeeding				
Yes	23	108.87(36.21)	4.68(-12.81-22.16)	0.594
No	30	104.20(27.25)		
Early infant formula feeding				
Yes	15	114.67(39.76)	11.77(-7.24-30.78)	0.219
No	38	102.89(27.04)		
Socioeconomic class				
upper middle socioeconomic	44	109.50(30.99)	19.278(-3.23-41.79)	0.092
low socioeconomic	9	90.22(28.72)		
Mode of delivery				
Spontaneous	11	92.64(26.61)	-17.15(-38.03-3.74)	0.105
Caesarean section	42	109.79(31.63)		
History of infants allergies				
Yes	7	120.86(23.59)	16.86(8.38-42.09)	0.186
No	46	104.00(31.83)		

*Independent sample T-test MD: mean difference C.I: Confidence Interval

birth. The evidence from this study will strengthen the opinion that zinc regulation is long-term, so that further studies are needed [17]. The tight regulation of systemic and cellular zinc involves 30 types of protein enzymes including ZnT, and ZIP. Meanwhile, no mediators or blood proteins that transport zinc from one organ to another have been found. This is different from iron, where the blood iron binding protein has been found, namely Hepcidin. Serum zinc is only 0.1% of the total body zinc where levels are influenced by absorption in the intestine, intestinal lumen zinc concentration and body needs. Serum zinc levels can change or fluctuate at any time because it is influenced by many factors. Thus, it is difficult to find evidence that zinc levels measured once (at 3 years of age) are associated with current allergy symptoms [17].

Stimulation on DC cells, T cells, and B cells by exposure to infections, allergens, and cigarette smoke can affect zinc levels in infancy. There has been no previous study that has proven the relationship between umbilical cord zinc levels and serum zinc in toddlers except for the results of this study. This phenomenon may be a function of zinc homeostasis which adjusts the stimulus conditions received to maintain normal immune function. The important role of membrane proteins such as zinc transporter (ZnT) and zinc importer (ZIP) in controlling cytoplasmic zinc of DC cells, Th0 cells, and B cells is not yet fully understood. It is possible that zinc membrane proteins are strongly influenced by epigenetic factors (environmental exposure) since early life. This can be related to the emergence of allergic or autoimmune disease phenotypes [18].

Other studies had only proven the correlation of zinc levels with total/specific IgE levels in school children such as the research in Korea [11]. The reason why there was a positive correlation of infant IgE with cord zinc is that allergen sensitization in the first 4 months is affected by neonatal zinc levels but we do not know why higher neonatal zinc levels are accompanied by higher infant total

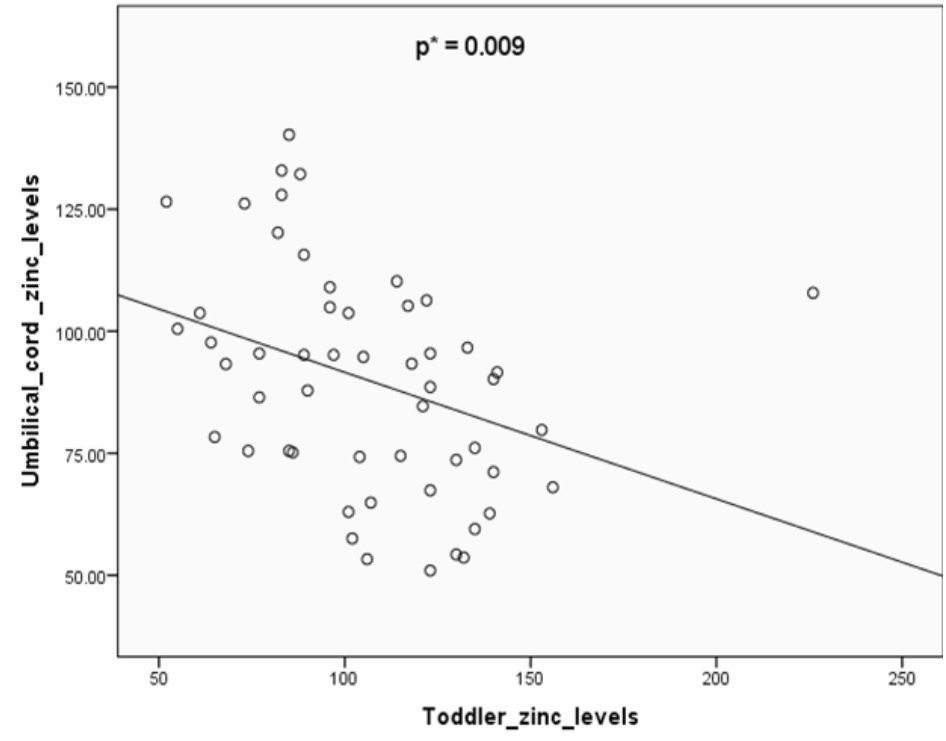

Figure 1. Correlation of umbilical cord zinc levels with toddler zinc levels. The graph shows a significant negative correlation between umbilical cord zinc levels and toddler zinc levels, r = - 0.354, p = 0.009. *Pearson correlation test.

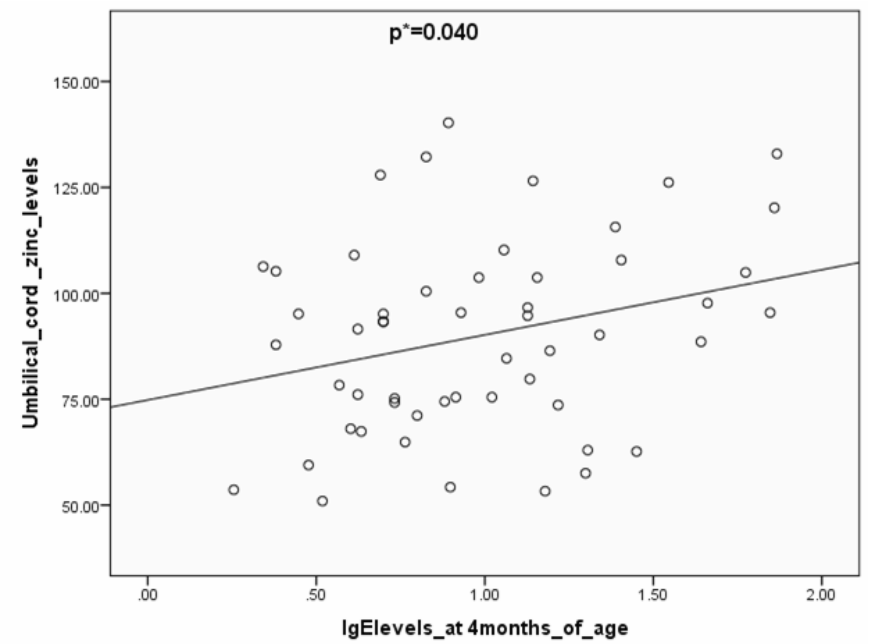

Figure 2. Correlation of infant IgE levels with umbilical cord zinc levels. There is a positive correlation between infant IgE levels and umbilical cord zinc, r = 0.282, p = 0.040. *Pearson correlation test

IgE levels as well. It should be noted that infants with high total IgE levels do not always have allergic symptoms both in infancy and at the age of 3 years. The results of the study found that zinc levels of toddlers with a history of high total IgE tended to be lower than controls. It is necessary to analyze whether allergen exposure factors affect zinc levels in toddlers. Previous research information has shown a correlation between serum zinc and total IgE in adults with atopic asthma. In this case there was an increase in IFN-γ and a decrease in IL-10 in PBMC cultures that have been stimulated with zinc [19]. Zinc can act as a mediator in allergic inflammatory reactions. Aggregation of FcεRI in mast cells is accompanied by rapid zinc release. However, this mechanism of IgE-dependent zinc release is not the same as degranulation [20].

FoxP3 expression in toddlers was not associated with health history in the first 3 years of age; also zinc levels were not associated with FoxP3 (Treg) expression in toddlers. Previous studies have not consistently proven the relationship of zinc with Tregs. Zinc has been shown to strengthen the T helper 1 cytokine (IFN-γ) [7]. Meanwhile, other studies have shown that zinc increases the presence of regulatory T cells while increasing IFN-γ [21]. Likewise, another study found that zinc supplementation stimulated $CD4^{+}CD25^{+}Foxp3^{+}$ Tregs but suppressed IFN-γ instead [22]. So the effect of zinc in stimulating the presence of Tregs is not consistent.

Foxp3 expression was higher in infants who were very rarely infected, although not significantly. It is known that exposure to infection stimulates Th1 cells [6, 7]. Thus, it can be explained that FoxP3 expression was higher in the group with very rare infections. This could be related to a lower Th1 cell pattern.

Treg cells always maintain the immunotolerance of the immune system. This can explain why there is no difference in Foxp3 expression in the two groups based on a history of infection, acute respiratory infection, and other medical history from infancy to 3 years. Treg cells have the role of maintaining immunological tolerance to various environmental stimuli (infections, allergens, cigarette smoke and others). Foxp3 regulates the gene expression program of Treg cells. The important role of epigenetic factors in determining the phenotype of the human immune response should also be considered [23].

The results of another study proved that prenatal exposure (cigarette smoke, disinfectant) in pregnant women was associated with a decrease in the number of umbilical cord blood Tregs. Also children with low umbilical cord Tregs had a 1.55 times risk of suffering from atopic dermatitis [24]. The subjects of our study were different because most toddlers had been exposed to cigarette smoke since birth (74%), so it was difficult to find the determinants of the effect of exposure to cigarette smoke on FoxP3 expression.

The limitation of this study is that many subjects dropped out and their specific IgE levels were not measured. Another limitation of this study is that it did not perform FoxP3 analysis in peripheral blood mononuclear cell cultures, which better reflect true T cell activity.

CONCLUSION

Cord zinc levels were lower in toddlers with allergic symptoms. In addition there was evidence of a negative correlation between zinc levels in infants and umbilical cord zinc. This fact may be related to the homeostatic mechanism of the human immune response that maintains normal balance through regulation of intracellular zinc such as ZnT and ZIP. There was no relationship between Foxp3 and zinc expression at the age of 3 years based on the medical history since birth. Zinc levels in early life may be more influential on the development of the immune response than the zinc at old age. Further studies are needed to prove that early life zinc has major effects on T cell proliferation-differentiation in the thymus and secondary lymphoid organs. In addition to zinc, research on the role of weaned foods with high fiber in preventing allergy symptoms is also necessary. The results of our research proved that early weaning food has no effect on allergies in infants and toddlers.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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