Original article:

The Low umbilical cord Zinc levels Lead to Atopic Allergic Infants : a Cohort study during 0-4 months of Age

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Abstract:

Background: The research on association between differentiation of Th1 and Th2 were previously conducted on experimental animals. The study on association between umbilical cord zinc and the risk of allergy symptoms during early stage of life has not been conducted in Indonesia. Studies on zinc and allergies are commonly investigated during adulthood and the results are still contradictive. ***Objective****:* The aim of the study is to determine the extent of zinc's role in the emergence of atopic allergy symptoms during the first 4 months age ***Methods****:* This prospective cohort study includes consecutive 80 healthy newborns followed up for 4 months after birth at Semarang Sultan Agung Islamic Hospital and Bangetayu Primary Health Care. Hypothesis testing were analyzed with unpaired t-test , chi-square test or fisher's test ***Results:*** The mean value of cord blood zinc in allergic infants were significantly lower when compared with non atopic allergy (74.1 ± 17.3 µg / dL vs 91.5 ± 22.6 µg / dL, p=0.029). Very early formula feeding were 4.4 times more at risk of suffering from allergies(p=0.023).***Conclusion:*** Umbilical cord zinc levels are associated with atopic allergy symptoms for the first 4 months of life. Formula feeding before 1 month of age was associated with atopic allergic infants

Keywords: umbilical cord zinc levels; atopic allergy; very early formula feeding

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Introduction

Relapsing allergy diseases can increase medical costs, reduce the quality of life of infants and children. In 2013, the prevalence of asthma in Semarang municipality were 4.5% which was higher than the asthma prevalence in Indonesia (5.3%), as well as increase in the incidence of asthma among infants under one year old.[1,2] Allergy symptoms that appear during the early life can cause disruption development of organ systems such as the respiratory tract system, gastrointestinal tract system, and balanced immune response. [3] Prevention based research of allergies preventions are needed to make bigger hopes for further generations to prevent its symptom in the future offspring.

Allergy is affected by genetic and environmental factors. The appearance of allergy symptoms is based on immune respond pattern that is tend toward to Th2. Th2 cells increases the sensitization of specific IgE production to certain allergens. [3,4,5,6] The development of immune response balance begins in early life, marked by the tendency of proliferation of Th0 cell differentiation towards the subset of Th1, Th2, or Th17, and T regulator as a factor

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to maintain immunotolerance.[5,6,7] Recently, trace elements including zinc have been studied for their role on allergy. Intracellular Zinc (Zn) is associated with an allergic response because it contributes in the proliferation of lymphocyte cell differentiation, cytokine release in dendritic cells (DCs), mast cells, B lymphocytes, and macrophages. In allergy, zinc is often associated with DC maturation and T cell expansion. [8,9]

The natural history of the earliest allergic disease appears is AD (atopic dermatitis) and food allergies, is then followed by allergic rhinitis and bronchial asthma, clinical manifestations from an early age can be persistent a few years or recover after getting older.[10,11] There were no published studies on zinc of umbilical cord in Indonesia. Previous similar study suggested that zinc levels are higher in the group of infants with 1-year-old wheezing symptoms.[12] The finding was contradicted to other study result that showing the high quartile of zinc levels is followed by a decrease of 5x prevalence ratio of asthma compared to control and lower zinc level was followed by an increase in the prevalence ratio of asthma of 2.5 times in adolescents.[13]

Studies on the umbilical cord zinc levels in neonates associated with the risk of allergic symptoms development in early age are not yet conducted in Indonesia. Allergic studies have focused more on population of older children. In addition, the findings of the previous studies were inconsistent. Studies on umbilical cord zinc levels of newborns are needed because it represents early life and maternal zinc levels indirectly. [14,15] This study aimed to investigate further the role of zinc towards the immune response since early life. Zinc levels of the newborn were represented by umbilical cord zinc level then it is observed approximately during the first four months on the possibility for arising allergic symptoms.

Methods

This research using prospective cohort study, observing newborns to approximately 4 months old of age or minimum during their 16 first weeks of life. Umbilical cord blood were drawn from the newborns in Sultan Agung Islamic Teaching Hospital and Bangetayu Community health center Semarang, between September to November 2017. Samples were taken consecutively.

Subject

This study was part of the newborn cohort study to determine the risk factors and breastfeeding protective effects on the development of allergies on under 6 months old. Observations on eligible 96 newborns with their parents agreed to sign informed consent, 16 drop outs (10 moved to a different place, 6 refused to follow further observations). Inclusion criteria include the full term healthy newborn, normal birth weight ≥ 2500 g up to 3900 gram, minimum were breastfed during the first month. The exclusion criteria were infant in hospital wards for high risk of severe illness, congenital syndrome suspect, moving far away from Semarang, the mother suffers severe illness in the Intensive Care Unit.

The definition of allergic infant is infant with symptoms and signs of allergic (atopic dermatitis, recurrent chronic cough, recurrent wheezing) accompanied by evidence of sensitization of specific allergen from skin prick test.[10,11] Symptoms and signs of atopic dermatitis (AD) were defined as itchy and rash symptoms on the cheek and around the neck in anamnesis based on questionnaires modification from ISAAC (International study of asthma in childhood), and physician's physical examination according to Hanifin and Rajka criteria simplified by UK labor party. Symptoms of allergic rhinitis, bronchial asthma anamnesis based on ISAAC modification questionnaires, and the definition by Respirology Task Coordinating Unit of Indonesian Pediatric Society. Allergy symptoms were confirmed by a physical examination by a doctor. Suspect of Bronchial Asthma was defined as recurrent chronic cough (cough> 2 weeks and recurrent cough in the last 3 consecutive months), or recurrent wheezing (wheezing with as cough or cold > 2 weeks, and relapse at least 2 episodes in the last 3 months). Allergic rhinitis was defined as recurrent sneezing and often cold without fever more than 1 month. All the symptoms of the allergy were added with a history of improvement in clinical symptoms with corticosteroid and bronchodilator therapy, and a history of parental complaints about the baby's sleep disturbance at night. [16,17,18]

Short duration of breastfeeding if the breastfeeding were given only until <2 months. Very early formula feeding if the formula milk were given before 1 month old. Non exclusive breastfeeding if formula milk was

given before 6 months old, early weaning food is the first solid food given before 4 months old. High risk level of allergy (family history of allergies) based on allergy detection card from Immunology Allergy Indonesian Pediatric Society, high risk allergy was defined as high or moderate scores with positive Skin Prick test for the mother.[19]

The dependent variables were newborns with and without allergic symptoms, whereas the independent variable was zinc umbilical cord level. Confounding variables include maternal age, gestational age, nutritional status of pregnant women, birth weight, high risk level of allergy, and external variables included nonexclusive breastfeeding, early weaning foods, short duration of breastfeeding and very early formula feeding.

Bivariate analysis used Fisher's alternative test because it does not meet the requirements of Chi square, confounding and external variables were controlled by chi square analysis. The hypothesis test of difference of mean of zinc level between two groups using unpaired T test, applied due to the normal distribution of data. The statistical test was considered to be significant at $p < 0.05$. The data were statistically analyzed using IBM SPSS Statistics V21.0.

Measurement of Zinc level of umbilical cord of neonates

Newborn zinc levels were taken from the baby's umbilical cord soon after birth, and the serum was taken with centrifuge before stored at -20C for analysis. The assay was conducted in the Iodine Deficiency Disorder laboratory Faculty of Medicine, Diponegoro University, Semarang. Zinc concentration was evaluated using atomic absorption flame emission spectrophotometer (AA-6401F Shimadzu, Japan)

Ethical consideration

All Parents of the baby were agreed to sign informed consent. The approval was obtained from Ethical Ethics Commission Faculty of Medicine Diponegoro University, Semarang (No. 555/EC/FK-RSDK/VIII/2017)

Results

A total of 96 newborns were included. Ten were dropped out because of moving to another city, 6 refused to follow up. During the 4 months study, 14 infants had allergic symptoms, but only 9 infants had positive skin prick test result (3 infants had atopic dermatitis and 4 had recurrent chronic cough symptoms, and 2 recurrent wheezing).

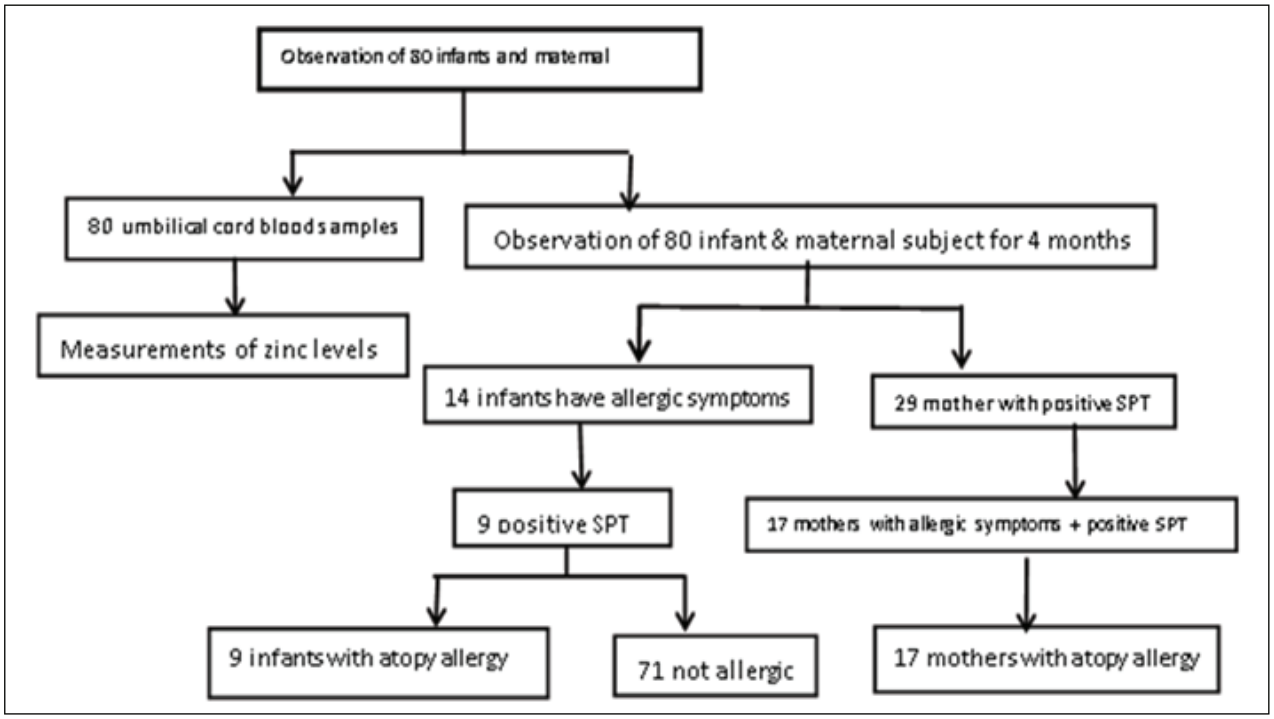


Figure 1.Research flow chart

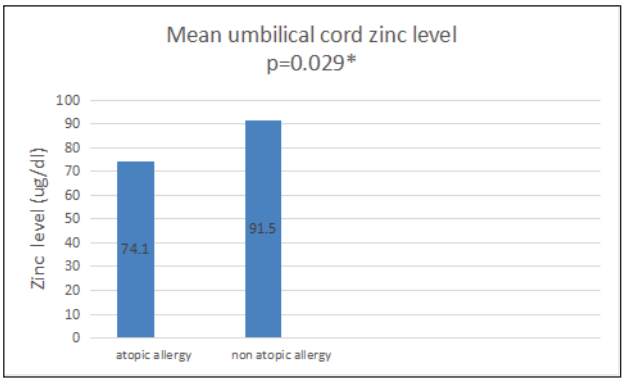
Table 1. Characteristics of Respondents and the Allergic Risk factors

Infants with Allergy	**infants without Allergy**					
	n	%	n	%	P*	RR
Mothers 'age (years)						
Young (17-35)	9	11.3	59	73.8	0.342	0.9
Old (> 35)	0	0	12	15		
Delivery method						
Cesarean section	7	8.8	58	72.5	0.674	1.23
Spontaneous	2	2.5	13	16.3		
Week of pregnancy (weeks)						
37 - 39	6	7.5	50	62.5	1.0	0.86
> 39 - 42	3	3.8	21	26.3		
Gender						
male	6	7.5	35	43.8	0.483	1.9
female	3	3.8	36	45		
MUAC						
- malnutrition(<23.5 cm)	1	1.3	13	16.3	1.0	0.59
- normal	8	10.0	58	72.5		
Birth weight						
2500 - 3000 g	5	6.3	37	46.3	1.0	1.13
> 3000 - 3900 g	4	5.0	34	42.5		
High risk level of allergy						
- Yes	3	3.8	14	17.5	0.392	1.85
- No	6	7.5	57	71.3		
Non-exclusive breastfeeding						
- Yes	7	8.8	30	37.5	0.073	4.07
- No	2	2.5	41	51.3		
Very early formula feeding						
- Yes	6	7.5	19	23.8	0.023	4.40
- No	3	3.8	52	65.0		
Short duration of breastfeeding						
- Yes	0	0	5	6.3	1.0	1.14
- No	9	11.3	66	82.5		
Early weaning food						
- Yes	2	2.5	9	11.3	0.603	1.79
- No	7	8.8	62	77.5		

* Fisher Test

Mid-upper arm circumference (MUAC)

Based on Table 1 , it is illustrated the characteristics and risk factors of respondents, there is no significant difference (p> 0.05) between allergic and non-allergic infants, meaning homogeneous research subjects, all risk factors for allergy can be controlled, except very early formula feeding associated with allergic infants (p:0.023, RR :4.4).



* Unpaired t test

Figure 2. Zinc level between two groups

Allergic infants have lower levels than without allergic atopy (74.1 ± 17.3 versus 91.5 ± 22.6, p = 0.029)

Figure 2 showed a significant difference in mean umbilical cord zinc levels with and without allergic (p=0.029<0, 05). Mean neonatal cord zinc level of infants with allergic (74.1 µg / dl) was lower compared with that of without (91.5 µg / dl with p = 0.029)

Discussion

This research was conducted by observing subject since birth to 4 months old in effort for early detection and immediate handling so it can prevent the allergic diseases progression. The younger the age of environment stimulation by aeroallergens (HDM, pollen, grass), RSV, pollutants (cigarettes smoke, gas) to the immature mucosal epithelium tends to the release the fibro genetic growth factor which is Epithelial-Mesenchymal Trophic Unit (EMTU) by the epithelium itself. EMT has the tendency towards pathological *remodeling* compared to the normal improvement, so the baby grows to suffer from heavy asthma bronchial after childhood or adult.[3] The role of zinc strengthens the structure and functions of respiratory and intestinal membrane barrier.[20]

Our research proved that there is a correlations between atopic allergic infants and umbilical zinc levels, where the levels were lower compared to the infants without atopic allergy symptoms. Contrary to the previous study, the umbilical zinc levels were higher in 1 year old infants with wheezing group.[12] The difference in dietary intakes between Indonesia and other countries are likely to make this study differs with the previous one. The umbilical zinc level in this research were lower compared to the previous study in Shenyang, China. Indonesia is considered area with zinc deficiency based on short stature nutritional problem and the community dietary habits that contain low amount of zinc. [21,22]

This research proved that the confounding variable has the impact which is formula milk sensitization on less than 1 month infants, while previous research was the effect of cigarette smokes exposure on group with allergic symptoms babies.[12] Formula milk causes commensal microbial dysbiosis. The intestinal microbiota dysbiosis will lower the mucosal resistance so that it will be penetrated by foreign antigen macromolecules which can cause allergens sensitization which initiate the Th2 development. The result of breastmilk's oligosaccharides fermentations by commensal microbes generate metabolite products that benefit the resistance of intestinal epithelium such as short chain fatty acids(SCFA). SCFA indirectly stimulates the formation of AMP (antimicrobial peptide) and increases the mucus production through the IL-22 stimulation pathways by Innate Lymphoid cells (ILCs), so the pathogenic bacteria and allergens can not easily penetrate the intestinal epithelium. The oligosaccharide of the breast milk stimulates the growth of Lactobacillus and Bifidobacterium in the intestines of the newborns. [23,24]

Despite the atopic genetic factor influence the allergy on babies, the early life environmental is considered as major role in triggering the onset of early allergic symptoms.[7] This research proved that zinc trace element factors are associated with infants' allergy, whereas high risk factors from atopic family are not associated with 4 months infants atopic allergy symptoms. [7]

Zinc level can affect the production and signaling of inflammatory cytokines of various types of immune cells. The level can decrease during acute phase response to stress, infection and trauma. Zinc flow is needed for protein synthesis, neutralization of free radicals and preventing microbial invasion. Zinc is an important component of the resistance of the intestinal epithelium, hypozincemia causes excessive neutrophil migration through damaged mucous barrier membrane. Zinc is needed to maintain the resistance of Adherens junctions (AJ). Hypozincemia activating proteolysis of E-chaderin dan β-catenin protein molecules belongs to Adherens junctions, this situation leads Tight Junctions and AJ more open and permeable including neuthrophills proinflammatory which damages the intestinal mucosa and respiratory epithalium.[25]

The increasing allergic prevalence in developing countries like Indonesia, generates thoughts that the effects of behavior changes and environmental exposure are highly associated with allergic diseases, Indonesia is one of the area at risk for zinc deficiency, and awareness of zinc deficiency cases must be higher. Mild zinc deficiency are often the impact is not only on child development, infections,

but also immune responses disorders such as allergy. Infants and children are vulnerable group because of the increasing needs of growth and developments compared to adults.[7,22,27]

Previous studies have indicated that stimulation of FcƐRI on mast cells causes degranulation and zinc release. Zinc release is similar to mediator of allergy inflammation.[27] whether this causes the decrease of zinc levels in allergic individuals especially during the asthma attack. Administration of zinc will decrease eosinophils from respiratory tract, because zinc act to inhibit the proliferation of eosinophils, furthermore, zinc increases Treg cells and release IFN-γ mediator (Th1), so that it inhibit the initial differentiation of cell Th0 to Th2, as stated by Prasad.[8,27]

Studies on birth zinc are very important to assess the potential of allergies since the early stage of life, our research finding supported the previous in vitro study that zinc can prevent the allergent sensitization by increasing Treg and IFN-γ cells expressions, thus limiting the Th2 develompent.[27] The zinc level of umbilical cord give the clear picture of the role of the newborn's zinc to the initial balance of early life's immunity, the possibility is that if the initial zinc level during the early life is in adequate amount or normal will prevent the allergy symptoms in the high risk atopic family individuals as well as the low risk individuals. [8,25,28]

The environment during conceived/ birth will give major impact on the ratio of Th1/Th2/Treg. [28] Moreover, our research did not prove the association between cigarette smoke exposure and the appearance of allergy during the first 4 months of age, while the other confounding and external variables were successfully excluded as predisposing factor of allergy. As in cohort study conducted in Jakarta, there was no association with cigarette smoke exposure with atopic dermatitis.[29] Furthermore, formula feeding before 1 month of age have high risk in atopic allergy on 4 months of age infants. Formula milk is suspected as the cause of dysbiosis which ultimately causes the resistance of the weak intestinal mucous layer, furthermore the low zinc level increases the risk of increased permeability of TJ and AJ intestinal epitellium which makes easier for antigenic macromolecules/ allergens to enter into systemic and trigger allergy sensitization. [23,25]

Considering several agenda of *Sustainable Development Goals*(SDGs) in Indonesia, goal number two is to end hunger, food security and nutrition improvement. All of them are highly associated with malnourishment prevention, including breastmilk adequacy and zinc micronutrients according to the theme of this study.[30] The research on zinc is very important because short stature is considered as nutrition problem in Indonesia which is associated with zinc micronutrients deficiencies in addition to the lack of animal protein intake.[22]

Study conducted on aeroallergen sensitized mice until experiencing acute and chronic inflammation, the zinc administration can reduce eosinophilia in bronchoalveolar lavage fluid in both acute and chronic inflammation model and lymphocytes in acute and chronic models. Zinc supplementation can also alter the zinc expression transporters, namely ZIP1, ZIP3, ZIP6, ZIP14 which decrease during sensitization with aeroallergens.[31]

The limitation of our study is on the numbers of confounding variables, eventhough it has been controlled by cohort, inclusion/exclusion criterion and analysis methods. Another limitation is the period of this study due to the individuals with the total high IgE serum does not immediately present the clinical symptoms of allergy, it may be necessary to conduct more sensitization until the clinical manifestations presents. In our study, all infants with atopic allergies have total IgE levels within the normal range. Individuals with high total IgE results are not always accompanied by allergic symptoms. There is no association between the levels of IgE total and the allergic complaints appearance except on bronchial asthma[31-34]. Future research requires a longer cohort observation, in addition to the zinc umbilical cord, breastmilk zinc check is also necessary and completed with zinc level examination during 4-6 months of age.

Conclusion

Low umbilical zinc levels trigger symptoms of atopic allergy during the first 4 months of life. Formula feeding before 1 month of age were associated with the clinical manifestation of allergic infants.

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Conflict of Interest

The Authors declared there is no conflict of interests.

Individual Contribution of the Authors:

Conceptual work: Priyantini S, Suprihati , Widyastiti NS , Soemantri

Data collection: Priyantini S, Suprihati

Manuscript writing: Priyantini S, Suprihati , Widyastiti NS , Soemantri

Editing of final manuscript: Priyantini S, Suprihati , Widyastiti NS , Soemantri

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