Stunted Toddlers Had Lower Hair Zinc Level Compared to Their Normal Peers: Result from a Case Control Study in Nganjuk

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Summary  Stunting is highly prevalent, including in Indonesia. Zinc intake and zinc status contribute to the etiology of stunting. However, zinc status measurement is not easy. Hair zinc level reflects long term exposure to zinc. This study aimed to analyze the difference in hair zinc level of stunted toddlers as compared to normal toddlers. This case-control study was conducted in Nganjuk with 23 stunted toddlers and 23 normal toddlers selected using simple random sampling. Hair zinc level was measured using atomic absorption spectrometry conducted at the Center of Research and Industrial Consultation Laboratory, Surabaya. The inclusion criteria for this study were children aged 24–59 mo, lived in Puskesmas Wilangan work-areas, and had uncoloured hair with minimum length of 1.5 cm; the list of respondents gathered from EPPGBM website, where it provides list of informations consist of data of malnutrition children. The study revealed that mean hair zinc level among stunted toddlers were 146.21 ± 16.83 mg/L while the hair zinc level among the normal toddlers were 157.07 ± 23.11 mg/L. There was significant difference in hair zinc level between stunted toddlers compared to their normal peers. Conclusions: Hair zinc level was associated with stunting and can be considered as biomarker for stunting.

Key Words  short stature, stunting, zinc level, hair, biomarker

Stunting is a worldwide nutritional problems (1) including in Indonesia. According to National Health Survey in 2013, the prevalence of stunting was 37.2% (2). Stunting is mainly caused by the inadequate nutritious food intake and infectious disease. The imbalance supply of nutrients and the energy expenditure will disturb cellular and organs growth to function normally. Micronutrients deficiencies such as iron, iodine, zinc, and vitamin A contribute to the etiology of stunting. Meanwhile, macronutrient deficiency of protein gives major contribution in child growth retardation. Long-term severe protein-energy malnutrition leads to severe malnutrition diseases such as kwashiorkor and marasmus.

Study showed that higher stunting rate was found in rural areas (2). Rural children have lower proteins and fats intake (3). Moreover, lack of essential mineral intake also contributes to poorer micronutrients status. Lower level of socioeconomic condition in rural areas might be the cause of lack access to more nutritious food products (4). Nganjuk, a small subdistrict in East Java, is one of many districts that were included in the priority areas for stunting reduction programs. The prevalence of stunting in Nganjuk was 44.3%, the highest prevalence in East Java province (5).

Zinc is an essential micronutrient for bodily functions and it is critical to various basic molecular functions (6). The major basic functions of zinc could be grouped into three functions: catalyse enzymatic activity, contribute to protein structure, and regulate gene expression. Zinc deficiency is frequently associated with malnutrition and affects about 1/3 population of the world (7). Zinc deficiency may cause growth retardation, tastebud dysfunction and loss of appetite. In regard of its effects on stunting, the causes and etiology, include in the following: nutrition (energy, macronutrients, micronutrients, and toxic factors); infection (injury to gastrointestinal mucosa); and mother-infant infection (8). Reflecting on zinc function, as an essential micronutrient for growth, zinc depletion of the organism thus virtually affects any organ system in the human body, and it encompasses a number of diverse biochemical changes resulting in a generalized metabolic dysfunction (6). It is supported with several studies have found that zinc deficiency has been associated with growth faltering and stunting (8).

Several studies argued on indicating the reliable and accurate method in zinc status assessment (9). The most widely used indicators are plasma zinc serum, followed by hair zinc status (10), and 24-h urinary zinc excretion as potential biomarker (9).

Some evidences discovered that low zinc concentration in children hair, reflected the zinc intake. Therefore, determining zinc status by hair zinc level is one of biomarker to assess zinc status in the body (10). Previous study used this method to assest children aged 6–9 y, zinc status (10). As zinc concentration remains constant in hair, it represents zinc accumulations for over 2–3 mo. Hence, hair zinc analysis represents zinc intake in the past and can be used as nutritional status measurement (10).
This study aimed to measure hair zinc level in stunting and non-stunting children aged 24–59 mo in Nganjuk.

MATERIALS AND METHODS

**Samples.** The present study was undertaken in Nganjuk subdistrict in East Java, Indonesia. Puskesmas (Health Center) Wilangan was purposively chosen to select the study subjects. Firstly, the list of respondents gathered from EPPGBM website, where it provides list of informations consist of data of malnutrition children. We make sure the total number of respondents in the selected location was more than 49 children based on EPPGBM data. Thus, there would be no issue regarding the lack of respondents.

The total stunting children aged 24–59 mo in the UPTD Puskesmas Wilangan was 71 children. The inclusion criteria for this study were children: aged 24–59 mo, lived in Puskesmas Wilangan work-areas, and had uncoloured hair with minimum length of 1.5 cm. Children who met the criteria then selected randomly by simple random sampling technique.

Children height were measured using microtoice with 0.1 cm precision. Details of this method was given in Gibson (12). Subsequently, children were categorized into stunting or not stunting based on.

We collected dietary data from the stunted and non-stunted control group using the interactive 24-h food recall and FFQ method. Two methods were performed to prevent bias measurement. Food recall aims to illustrate respondents’ daily intake whereas FFQ to explained daily intake pattern. Hair samples for zinc measurement were collected by trained professional barber. Hair was cut from the back-part of the head (occipital) in 1.5 cm length, from the tip of the hair. Afterwards, all of hair samples sent to the Center of Research and Industrial Consultation Laboratory, Surabaya for zinc measurement using Atomic Absorption Spectrophotometry (AAS).

**Statistical analysis.** We inputted the data using WHO Antro 2005 to calculate the height-for-age. For statistical analyses, SPSS 21.0 were used. Descriptive statistics included to measure the characteristics of respondents, zinc intake adequacy and hair-zinc status. Data displayed as mean percentage and frequency.

Three-days 24 h recall with non-consecutive days were completed then we categorized the result into deficit (<75% RDI) and normal (≥77% RDI). Subjects’ zinc intake was taken and compared with the recommended daily intake from Indonesian Health Ministry data in 2013.

Chi-square used to test the nominal or ordinal data scale but if it does not meet the requirements then Fisher Exact test is used for age, sex, birth weight, premature events, history of exclusive breastfeeding, and hair zinc status t-test independent scale used for numerical data with normal data distribution (p<0.05), otherwise for abnormal distribution Mann-Whitney is used (p<0.05). Kolmogorov-Smirnov test used to test data normality. Chi-square, independent t-test, and logistic regression with p<0.05 and confident interval 95% were used to find the correlation and differences in each variable.

**RESULTS**

The characteristics of children in this study are pre-

| Variables                      | Categories             | Stunting | Non-stunting | p-value* |
|--------------------------------|------------------------|----------|--------------|----------|
| Age                            |                        | n        | %            | n        | %        |          |
| Toddlers (24–36)               |                        | 7        | 30.4         | 7        | 30.4     | 1.000    |
| Pre-schoolers (37–59)          |                        | 16       | 69.6         | 16       | 69.6     |          |
| Gender                         |                        |          |              |          |          |          |
| Male                           |                        | 9        | 39.1         | 9        | 39.1     | 1.000    |
| Female                         |                        | 14       | 60.9         | 14       | 60.9     |          |
| Birth History                  | Low-birth weight (LBW) | 3        | 13.0         | 1        | 4.3      | 0.608    |
| Normal weight                  |                        | 20       | 87.0         | 22       | 95.7     |          |
| Premature History              |                        |          |              |          |          |          |
| Premature                      |                        | 2        | 8.7          | 2        | 8.7      | 1.000    |
| Normal                         |                        | 21       | 91.3         | 21       | 91.3     |          |
| Breastfeeding Practice         | Exclusive              | 13       | 56.5         | 12       | 52.2     | 0.767    |
| Not exclusive                  |                        | 10       | 43.5         | 11       | 47.8     |          |
| Total                          |                        | 23       | 100.0        | 23       | 100.0    |          |

Based on Chi-square test, α=0.05.
Hair Zinc Level of Stunted Toddler

The distribution of zinc intake adequacy and other nutrients such as energy, protein, and iron are illustrated in the Table 2. As can be seen, most of stunting children consumed zinc (52.2%) less than the RDI whereas normal children (95.7%) had enough. In comparison with others nutrients intake, stunting children experienced roughly similar patterns, with inadequate energy and iron intake.

Most stunting toddlers had less hair zinc level which was 65.2%. Statistical chi-square test with \( \alpha = 0.05 \) shows the value of \( p = 0.039 \) which concluded that there is a significant difference in hair zinc levels between stunting toddlers stunting and non-stunting (Table 3). The average of hair zinc levels in stunting toddler was 146.21 ± 16.83 mg/L, whereas in non-stunting was 157.07 ± 23.11 mg/L.

The Table 4 shows that there are significant differences based on energy, protein, zinc and iron intake between stunting and non-stunting, with \( p < 0.001 \).
The average zinc intake of stunting children was 3.08 ± 0.79 mg lower than the non-stunting which was 4.19 ± 0.71 mg.

**DISCUSSION**

The problem of chronic malnutrition in infants which is the effect of lack of food intake including lack of zinc can be identified through low hair zinc levels (10). These results are in line with studies that have been carried out in Surabaya which illustrate the concentration of hair zinc levels that are less (<150 mg/L), the majority of which occur in stunting toddlers (13). Likewise, by the results of Rahmawati and Wirawanni (10) in Kendal District in children aged 6–9 y showed the results there were differences between hair zinc levels based on the degree of stunting and there was a positive correlation between hair zinc levels with z-scores height/age, hair zinc levels increases with the increasing z-score height/age. The average level of hair zinc in stunting toddlers is lower than the non-stunting (14).

Research conducted by Oktiva and Adriani (2017) in toddlers aged 12–24 mo in Surabaya showed the opposite, there was no difference in hair zinc levels of stunting and non-stunting. Research with the same age target (12–24 mo) also conducted in Semarang showed the similar result, namely there was no relationship between hair zinc levels and nutritional status (length/age) (15).

Clinical signs of zinc deficiency in infants such as growth disorders can also cause appetite disorders (anorexia) which can be related to low hair zinc levels (<150 ppm) (12). Anorexia can result in a decrease in energy intake about 20% lower than in general conditions, this also affects the rate of synthesis of protein breakdown, decreases the absorption of fat and protein from food, causing a decrease in energy absorption from food compared to normal conditions (16). Zinc deficiency can also be associated with stopping hair growth (alopecia), besides that in the present study there were some toddlers who have thin and brown hair which is also due to the zinc deficiency (12).

To conclude, stunting children with poor zinc intake obtained lower hair zinc results compare with non-stunting children.

**Disclosure of state of COI**

No conflicts of interest to be declared.

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