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Quantitative Examination of Urine Zinc Levels in Normal Toddlers in Jakarta, Indonesia

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ABSTRACT : Background: Zinc deficiency causes approximately half a million infant deaths every year worldwide. Disease mortality caused by zinc deficiency in children is high, including diarrhea, pneumonia, and malaria. Zinc concentration in urine is one of important values to early detect zinc deficiency. Low zinc concentration in the body could decrease urinary zinc concentration until 96%. Data about mean of zinc concentration value in normal infant urine is not established. Methods: This study is a descriptive study with cross-sectional design. Samples are urine from 28 normal toddlers (2-5 years old) in Jakarta. Zinc concentration is measured by adding reagent 1-(2-pyrildilazo)1-naphtol (PAN) and base buffer solution ammonium chloride pH 10. PAN addition to zinc-containing solution under base condition will form color complex. The absorbance is read at 560 nm by spectrophotometer. Results: Subjects majority was male (n=21), normal nutritional status (n=20) and normal body height (n=26). The median of urinary zinc levels is 1.697 (11.242±0.182) mmol/L. We found that urine with higher density had high urinary zinc levels and urine with lower density also had low urinary zinc levels.
Conclusion: Mean value of urinary zinc levels in normal toddlers is 2.267 mmol/L.

KEYWORDS - Zinc levels, Toddlers, Urine, Indonesia

I. INTRODUCTION

Zinc deficiency is predicted to cause around half a million deaths under-five years children every year in the world.[1] There are 176,000 deaths of diarrhea, 406,000 of pneumonia, and 207,000 of malaria caused by zinc deficiency in under-five years children. Zinc deficiency prevalence is extremely high in many parts of the world, especially in regions with high mortality rate of diarrhea, pneumonia, and malaria such as Indonesia. Zinc deficiency causes immune system disruption, both innate and adaptive immune system. Therefore, zinc deficiency increases morbidity and mortality of infection such as pneumonia and malaria.[2] Based on Basic Health Research (*Riset Kesehatan Dasar*) in 2017, there are 21 diarrhea outbreaks in Indonesia occurred in 12 provinces with total surrenders 1.725 children and 34 deaths in a year. Under-five years children mortality rate of pneumonia in Indonesia is the second largest as much as 15.5% deaths of children. Meanwhile, based on subdirectory report of upper respiratory tract infection (Laporan Rutin Subdit ISPA) in 2017, pneumonia incidence per 1000 under-five reaches 20.54. Malaria incidence in Indonesia in 2017 is 0.99 per 1000 people.[3,4]

Zinc has roles in various body functions including enzyme cofactor, signaling molecule, transcription factor, and second messenger. Besides, zinc also has role in synthesis and secretion of some hormones mainly sex hormones and insulin. Zinc also acts as signaling molecule occurs in nervous system and immune system. In nervous system, zinc plays a role in proliferation stimulation, activation, and immune cells chemotaxis. Therefore, zinc deficiency can suppress many functions such as immunity, peroxidation, neuro-psychological function, nerve conduction, and child development.[5] Zinc deficiency is diagnosed from clinical signs and symptoms as explained before such as diarrhea, immune suppression, nerve conduction disruption and followed with laboratory examination. The laboratory examinations for zinc levels could be conducted using blood, hair and also urine. Zinc can be excreted in urine, so it can be used for zinc levels examination in urine. Daily zinc excretion in urine is 7 mmol (0.5 mg) in adults.[5] Urine zinc levels less than normal value indicates zinc deficiency. In addition, zinc levels more than normal value indicate zinc excess and some medical conditions such as catabolism increase, hemolysis, cirrhosis, and diabetes mellitus.[6] Zinc examination in urine is not commonly used in zinc deficiency diagnosis. However, urine sample collection is not invasive and easy to do. In addition, low levels of zinc in the body can decrease urine zinc level up to 96%. Therefore, urine zinc levels can be used as indicator of individual zinc status.

The most common laboratory examination used in zinc deficiency diagnosis is serum zinc level. Normal urine zinc level in toddlers (under-five years children) has not been know yet, therefore, further study is needed.[5], [7]

II. METHODS

This study is an observational descriptive study with cross-sectional design. This design is chosen to explore urine zinc level mean in normal under-five years children. Experimental urine samples are collected from community development program participants in Duren Sawit District, East Jakarta. Subjects are 30 normal under-five years children (toddler) without any symptoms of fever, diarrhea, and vomiting. Urine is collected for 24 hours by wasting first morning urine at 07.00 am on the first day of collection, all the urine excreted within the next 24 hours is collected in a jar and the final collection is morning urine in the next day at 07.00 am.

Reagent Preparation : Buffer pH 10 is prepared by dissolving 6 g of ammonium chloride in 20 mL of dH2O. This solution is added with 57 mL of 25% ammonia solution. The solution is then diluted with dH2O until the volume reaches 100 mL. The second step is preparing dissolving and magnesium masking solution by dissolving 10 g of SDS (sodium dodecyl sulfate) into 500 mL dH2O followed by 10 grams of ammonium fluoride and dH2O addition until the volume reaches 900 ml. The third step is preparing PAN (1-(2-pyryldilazo)1-naphtol) solution. The PAN solution is made by dissolving 10 mg of PAN in 100 mL methanol. The last step is preparing the reagent solution by mixing 100 mL buffer solution pH 10, 900 mL dissolving and magnesium masking solution, and 20 mL PAN solution.

Urine Zinc Levels Measurement : The principle of zinc levels measurement is 1-(2-pyryldilazo)1-naphtol (PAN) added will react with zinc in alkaline condition will form a color complex. This color complex can dissolve in water with sodium dodecyl sulfate (SDS) addition.[8] The color intensity is then measured using spectrophotometer. Three milliliter of reagent solution is poured into microtube. Then, 20 µL sample/standard is added into it. The mixture is then incubated for 5 minutes under room temperature. The next is light absorbance measurement using spectrophotometer in wavelength 560 nm.

Data Analysis : The collected data will be tested for normality using Saphiro-Wilk. If the data is normally distributed, data will be presented in mean. If the data is not normally distributed, the data will be presented in median.

III. RESULTS

The subjects of this study are under-five years children with range 2-4 years old. Total sample size is 30 children and two subject was excluded because not able to be identified. Gender distribution 22 (75%) are male and 7 (25%) are female. For nutritional status, about 20 subjects are normal (71%), 2 (6%) under-nourished, 2 (6%) severely under-nourished and 5 (17%) over-nourished. Subjects body height are 26 (94%) in normal stature, 1 (3%) in short stature, and 1 (3%) in tall stature. Based on urine density, 13 subjects (46%) have low density and 15 (54%) have high density (see Table 1).

Table 1. Characteristics of The Subjects of The Study

Variable	N	%
Gender		
Male	21	75%
Female	7	25%
Nutritional Status		
Normal Nourished	20	72%
Under Nourished	2	6%
Severely Under Nourished	2	6%
Over Nourished	4	16%
Stature		
Normal	26	94%
Tall	1	3%
Short	1	3%

Urine Density		
≤1.020	13	46%
>1.020	15	54%

The data of urine zinc levels is not normally distributed ($p<0.05$), so we presented the data in mean, median, standard deviation, minimum value, and maximum value are shown in table 2. We also compare urine zinc levels based on the urine density (table 3).

Table 2. The Result of Urine Zinc Level Measurement

Mean (mmol/L)	2.267
Standard deviation (mmol/L)	2.144
Median (mmol/L)	1.697
Minimum (mmol/L)	0.182
Maximum (mmol/L)	11.242

Table 3. Urine Density

Urine Density	n	Mean of Urine Zinc Level (mmol/L)	P value Mann-Whitney U Test
Low (≤1.020)	13	1.58	0.002
High (>1.020)	15	2.40	

IV. DISCUSSIONS

Daily water necessary for children aged 1-5 years old is about 100 ml/kg body weight. Water excretion from body is 65% through urine from total daily loss in children.[9] Based on this theory, daily urine volume in children weight 12 kg is about 1200 mL. Meanwhile, daily zinc loss in urine is about 15% from total daily zinc loss. Daily zinc loss in children age 1-5 years old is about 35 µg/kg/day, therefore daily zinc through urine is 5.25 µg/kg/day.[10] In children weight 12 kg, daily zinc loss is about 63 µg/day or equal to 0.063 mg/day. The result of the calculation of daily zinc excretion in this study is not in line with the theory. In this study, by referring to one of the subjects with urine volume 867 mL in 24 hours and median value of the zinc level of the study result 1.70 mmol/L, daily zinc loss is about 95.80 mg/day. This result shows that daily zinc loss of under-five years children in Jakarta is much higher than in the theory. This result is not in line with the theory may be because the volume of the urine is not fully 24 hours. This can decrease the validity of the study because the osmolarity of the urine is different from time to time within 24 hours. Urine after waking up in the morning has highest osmolarity. Besides, some conditions such as physical activity not followed by drinking enough water will also increase the osmolarity of the urine.

Some factors could influence the urine zinc levels, such as gender, urine pH, physical exercise, drinking and eating habits.[11] A study reported that males showed significantly higher zinc urinary levels than females.[12] Zinc excretion will increase in subjects with age up to 30 years. Urinary zinc excretion also significantly lower when the urinary pH more than 7. People with high consumption of protein, fiber, coffee, tobacco or alcohol also have high urinary zinc level, although there is a study reported that urinary zinc levels are not affected by smoking and drinking habits.[12] People with routinely physical exercise have depletion of urinary zinc level.[11] We found in this study that urinary zinc levels in line with urine density. If urine density is high, the urine zinc level will be found increase and if urine density is low, the urine zinc level will be found decrease. It is of course understandable that high urine density will contain higher solutes including zinc. We hope that our results of this study can be used as basic data for further research. because there are very few studies that reveal urinary zinc levels in children, especially toddlers, so these results can be used as a reference for further research.

V. CONCLUSION

We conclude that urinary zinc levels in normal toddlers (under five are years) is about 2.2676 mmol/L. We can use urine zinc levels to measure zinc levels in our body, because the sampling is non-invasive so it is suitable for toddler subjects.

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