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Original Article

Relationship between serum zinc and homocysteine in children with nephrotic syndrome

Welli Hamik, Dany Hilmanto, Sri Endah Rahayuningsih

Abstract

Background In children, most idiopathic nephrotic syndrome (NS) is a minimal lesion, which responds well to steroids. Hyperhomocysteinemia is pathologic and worsens NS by causing chronic inflammation, leading to glomerular sclerosis. Zinc metalloenzymes are involved in homocysteine metabolism.

Objective To assess for a possible relationship between serum zinc and homocysteine in children with NS.

Methods A cross-sectional study was conducted in children with NS aged 1-18 years, who were admitted to Hasan Sadikin Hospital, Bandung, West Java, from November 2017 - January 2018. Subjects were selected consecutively. Serum zinc and homocysteine were measured in all subjects. Statistical analysis was done with Pearson's correlation test. If the distribution was not linear, the analysis was continued with non-linear regression.

Results There were 23 children who met the inclusion criteria. Mean serum homocysteine and zinc levels were 10.37 (SD 4.11) μmol/L and 51.13 (SD 29.69) μg/dL, respectively. Pearson's correlation analysis showed no linear correlation between them (r coefficient -0.173; P=0.430). However, after adjusting for age and serum albumin level, multiple regression analysis suggested a cubical relationship between serum homocysteine and zinc, using the equation: homocysteine = -4.572 + 0.735 x zinc - 0.0012 x zinc^2 + 0.00005 x zinc^3 x age (months) (R^2 multiple=53.2%; P=0.012). This equation indicates that 53.2% of homocysteine variation was influenced by serum zinc concentration.

Conclusion In childhood NS, homocysteine is not correlated linearly with zinc, but related with cubical model. **[Paediatr Indones. 2019;59:98-103; doi: http://dx.doi.org/10.14238/pi59.2.2019.98-103**].

Keyword: *children; relationship; nephrotic syndrome; homocysteine; zinc*

Nephrotic syndrome (NS) is a common pediatric kidney disease characterized by leakage of protein from the blood into the urine. It remains a major cause for referral to pediatric nephrologists because of the chronicity and the complexities of the disorder.[1] Nephrotic syndrome can be classified into 3 groups: primary or idiopathic, if not accompanied by other systemic diseases, secondary to disease or other systemic conditions, and congenital NS.[1] As many as 90% of NS cases in children aged 1-10 years are idiopathic.[2] Most idiopathic NS in children are minimal lesions which respond well to steroid therapy.[3]

Focal segmental glomerulosclerosis (FSGS) is a further stage of such a minimal lesion.[3] In contrast to the minimal lesions that have not undergone structural changes in light microscopy, focal segmental glomerulosclerosis is characterized by segmental destruction of the glomerular capillaries, accompanied by adhesions formed between the sclerosis segment and Bowman's capsule.[3] As much as 80% of FSGS

From the Department of Child Health, Universitas Padjadjaran Medical School/Dr. Hasan Sadikin Hospital, Bandung, West Java, Indonesia.

Corresponding author: dr.Welli Hamik, SpA. Department of Child Health, Universitas Padjadjaran Medical School/Dr. Hasan Sadikin Hospital, Jl. Pasteur No.38, Sukajadi Bandung 40161, West Java, Indonesia. Email: welli_mu@yahoo.co.id.

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forms are resistant to steroid treatment and will develop into end-stage renal disease (ESRD). [3]

Clinical and epidemiologic studies in the last 20 years have shown a positive correlation between elevated homocysteine (Hcy) levels and ESRD as well as their cardiovascular complications.[4] Laboratory studies have shown that Hcy directly induces glomerular injury, affects glomerular endothelial cells, mesangial cells, and podocytes.[5]

Zinc is an important mineral for the human body and is known to have a role in Hcy metabolism. To date, studies on the effect of zinc on NS have been limited and to our knowledge no study has been done directly correlating zinc and Hcy in pediatric NS.[6] Two zinc metalloenzymes involved in Hcy metabolism are methionine synthase (MS) and betainehomocysteine methyltransferase (BHMT).[7] Both of these enzymes play a role in catalyzing methyl transfer in the homocysteine metabolic process.[8] The purpose of this study was to analyze for a correlation between serum zinc and homocysteine levels in children with NS.

Methods

This cross-sectional study was performed in children with nephrotic syndrome in the Pediatric Nephrology Division, Hasan Sadikin Hospital, Bandung, West Java, from November 2017 to January 2018. The inclusion criteria were children aged 1 to 18 years who were diagnosed with NS during the nephrotic stage. We excluded NS patients with Down syndrome, proliferative blood disorder, hypothyroidism, hyperthyroidism, diabetes mellitus, chronic kidney disease with glomerular filtration rate <60 mL/minute/1.73m^2, severe malnutrition, chronic liver disease, and patients receiving phenytoin or carbamazepine.

Subjects were taken consecutively until the required minimum sample size was met. Subjects' data included name, sex, age, weight, height, NS diagnosis, urea and creatinine levels, blood albumin levels, and serum zinc and serum homocysteine levels. Serum zinc level was measured by ICP-MS method using an Agilent 7700 instrument with required blood specimens of 250-750 μL. Serum homocysteine level was measured by chemiluminescent method using an Advia Centau tool, requiring blood specimens of 100-200 μL.

The normal range of serum zinc levels used in this study was based on age-dependent constraints with the following ranges: age <6 months: 26-141 μg/dL, 6 months to 12 months: 29-131 μg/dL, 1 to 2 years: 31 -201 μg/dL, 2-4 years: 26-116 μg/dL, 4 to 6 years: 48-119 μg/dL, 6 to 10 years: 48-129 μg/dL, 10 to 14 years: 25-148 μg/dL, and 14 to 18 years: 46-130 μg/dL. From the normal serum zinc values, we classified zinc <40 μg/dL as zinc deficiency for children above 4 years old.

With a 5% significance level and 80% power of the test, the coefficient of the relationship between x and y with r -0.55 yielded a minimum required sample size of 20 subjects. Statistical analysis was performed using Pearson's correlation test to determine the correlation between serum zinc and serum homocysteine levels. The type of relationship was determined by double regression analysis. Data analysis was performed using *SPSS version 21 for Windows* software. This study was approved by the Health Research Ethics Commission of Dr. Hasan Sadikin Hospital, Bandung.

Results

From November 2017 to January 2018, 23 study subjects met the inclusion criteria and no children were excluded. There was no significant difference in numbers of boys (48%) and girls (52%). The youngest subject was 24 months (2 years) and the oldest was 200 months (16 years and 8 months). The mean age of subjects was 161 months (13 years and 5 months). Most NS diagnoses were steroid-resistant NS (70%) followed by frequent relapse NS (17%), first attack NS (9%), and steroid-dependent NS (4%) (**Table 1**).

Table 2 describes the statistical analysis of the variables studied. Subjects' mean serum homocysteine level in this study was 10.37 (SD 4.11, range 5.3-19.5) μmol/L. The mean zinc concentration was 51.3 (SD 29.69, range 15-124) μg/dL. The mean blood albumin level was 1.46 (SD 0.99, range 0.3-3.5) g/dL. Shapiro-Wilk data normality test showed that the homocysteine data had a normal distribution (P=0.093).

Table 3 shows the correlations between variables studied. Pearson's correlation showed that serum zinc and serum homocysteine were not linearly correlated (r -0.173, P=0.430). For the linear model, age (months)

had a significant correlation with serum homocysteine levels, with the equation: homocysteine levels = 6.10 + 0.042 x age (months); with R^2=33.1%. This result indicated that 33.1% of variation in homocysteine levels was influenced by age (months)

Figure 1 shows the relationship between serum homocysteine and zinc levels in children with NS. After controlling for age and albumin, the relationship between serum zinc and homocysteine in NS patients was significant in a cubic model with the following equation: homocysteine = -4.572 + 0.735 x zinc - 0.0012 x $zinc^2$ + 0.00005 x $zinc^3$ x age (months) (R^2 multiple=53.2%; P=0.012). This result indicated that 53.2% of homocysteine variation was influenced by serum zinc, and 46.8% by other factors.

The distribution of serum zinc and homocysteine data showed that serum zinc levels below 40 μg/dL would not affect the levels of homocysteine and even tended to increase the serum homocysteine levels. Conversely, when zinc levels were above 40 μg/dL (higher serum zinc levels), serum homocysteine levels tended to be lower.

Table 1. Characteristics of study subjects

Characteristic	(N=23)
Sex, n (%)	
Male	11
Female	12
Age, month	
Mean (SD)	161.6 (56.2)
Range	23-200
Diagnosis, n (%)	
First attack NS	2
Frequent relapse NS	4
Steroid-dependent NS	1
Steroid-resistent NS	16

Discussion

This study provides information on serum homocysteine and zinc levels, as well as the correlation between them in children with NS. Hyperhomocysteinemia (Hhcy) was noted in 52% of our subjects. Similarly, a previous study showed an increase in Hcy levels and decreased vitamin B12 levels in 42 children with NS in Nigeria.[9] Hyperhomocysteinemia in NS patients was suggested to be associated with an inhibition of the homocysteine remethylation process or disruption in cysteine clearance.[9]

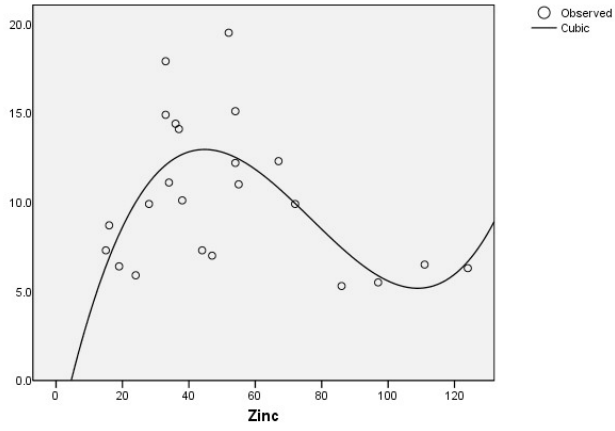

Figure 1. Diagram of the relationship between serum zinc and homocysteine in NS patients

Table 2. Serum zinc, homocysteine, and albumin levels in NS patients

Variables	Mean (SD)	Median (range)	Data normality test (P value*)
Serum homocysteine, μmol/L	10.37 (4.11)	9.90 (5.3-19.5)	0.093
Serum zinc, μg/dL	51.13 (29.69)	44.0 (15-124)	0.024
Albumin, g/dL	1.46 (0.99)	1.1(0.3-3.5)	0.007

Note: * Based on Shapiro-Wilk test, P value >0.05 normal data distribution

Table 3. Correlation between variables studied

Correlation	Correlation coefficient (r)	P value*
Homocysteine and age	0.624	0.001
Homocysteine and zinc	-0.173	0.430
Albumin and homocysteine	0.336	0.117

*based on Pearson's correlation test

The normal homocysteine value in adults is 5-15 μmol/L, but there is no consensus on normal levels in children.[10] Naseri *et al.* in his study on Hhcy in children and young adults on dialysis, defined it in children by age: >8.3 μmol/L for children aged 2-10 years, >10.3 μmol/L for children aged 10-15 years, and >11.3 μmol/L for children aged 15-18 years.[10]

Statistical analysis revealed that age (months) was linearly correlated with homocysteine levels, with the following equation: homocysteine level = 6.10 + 0.042 x age (months), with R^2=33.1%. This equation indicates that 33.1% of homocysteine level variation was influenced by age and was consistent with a study by De Laet *et al.* who observed that total homocysteine concentrations were lowest in younger children and increased with age.[11]

Hyperhomocysteinemia most commonly occurs in steroid-resistant NS. As many as 68% of our patients with steroid-resistant NS had Hhcy. This result was consistent with other clinical studies that showed a pathogenic effect of Hhcy that caused podocyte injury and glomerulosclerosis.[5,12] Hyperhomocysteinemia can cause injury and glomerular sclerosis due to impaired extracellular matrix metabolism, decreased protection from nitric oxide (NO), and increased reactive oxygen species (ROS).[13] Subjects' mean serum zinc level was 51.3 (SD 29.69, range 15-124) μg/dL, with zinc deficiency in 8 of 23 (35%) subjects. Similarly, Dwivedi *et al.* showed a decrease in zinc and copper levels in patients with NS.[14] The serum zinc level decrease in NS was associated with increased urinary zinc excretion through mechanisms of renal secretion or reabsorption. Other mechanisms for reduced zinc include nutritional deficiencies, low intake of zinc in the diet, as well as decreased absorption of zinc or increased secretion of zinc into the intestine.[8]

Zinc deficiency occurs most frequently in frequent relapsie NS patients, followed by steroid-resistant NS. No zinc deficiency was observed in the first attack NS patients. Previous studies by Arun *et al.* and Bhatt et al. showed that zinc supplementation may decrease the incidence of relapse in NS patients,[15,16] due to the effect of zinc in reducing the risk of infection, particularly infection of the gastrointestinal and respiratory tracts.16 Zinc deficiency causes down-regulation of Th1 cytokines, relative Th-2 bias, and increased risk of infection. Zinc supplementation strengthens IL-1 and interferon gene expression, thereby restoring the Th1 immune response. The balance of Th-1–Th-2 cytokines may prevent the occurrence of relapse in NS.[15]

Homocysteine did not linearly correlate with zinc in our study. After controlling for age and albumin level, homocysteine was observed to have a significant association with zinc by a cubic model. The equation for this model was homocysteine = -4,572+0.735 x zinc – 0.0012 x $zinc^2$ + 0.00005 x $zinc^3$ x age (months). The R^2 coefficient determinant of 53.2% means that 53.2% of the variation in homocysteine was determined by zinc.

Other factors that may affect homocysteine include genetic abnormalities such as homocysteinuria, cystathionine beta synthase (CBS), methylentetrahydrofolate reductase (MTHFR), and Down syndrome. In addition, physiological determinants such as gender, age, kidney function, and muscle mass, as well as lifestyle determinants such as coffee and alcohol consumption, smoking, exercise, as well as certain clinical conditions such as blood folic acid and vitamin B12 levels, hyperproliferative disorders, hypothyroidism, diabetes, and consumption of anti-seizure medicines may affect homocysteine levels.[17]

The distribution of serum homocysteine and zinc levels in this study showed that the zinc < 40 μg/dL had limited association with serum homocysteine levels, and even tended to increase the serum homocysteine. This finding was in agreement with a study on the effects of zinc deficiency and zinc supplementation on homocysteine levels and enzyme-related expression in rats. Jing *et al.* showed that zinc deficiency increased serum homocysteine levels and reduced mRNA levels of methionine synthase enzymes.[7]

Conversely, when zinc levels were higher (above 40 μg/dL), serum homocysteine levels tended to be lower. As such, zinc levels above 40 μg/dL may have a protective value against hyperhomocysteinemia. Previous studies have shown that zinc supplementation may help lower Hcy levels. Heidarian et al. observed that zinc supplementation in patients with type 2 diabetes mellitus with microalbuminuria decreased serum Hcy levels.[18] Jing *et al.* also showed significant negative correlations between serum homocysteine and zinc levels in rat liver and kidneys (r -0.632; P<0.01 and r -0.534; P<0.05, respectively).[7]

A possible pathomechanism to explain such a correlation is the presence of zinc metalloenzymes in Hcy metabolism, two of which are methionine

synthase (MS) and betainehomocysteine methyltransferase (BHMT).[7,17,19] Homocysteine is metabolized from the body via transulfuration and remethylation pathways.[7] In the remethylation path, homocysteine is converted to methionine and requires methylcobalamin as a cofactor and 5-methyltetrahydrofolate as the substrate. This process also requires methionine synthase (MS) to catalyze the methyl transfer of 5-methyltetrahydrofolate from Hcy.[7] Other remethylation pathways are regulated by BHMT, which catalyzes the transfer of methyl from betaine to Hcy to form dimethylglycine and methionine.[7,14,20]

A limitation of this study was that subjects of this study were not examined for genetic disorders such as CBS and MTHFR gene defects that could affect homocysteine levels. We also did not measure folic acid and vitamin B12 levels, which are currently the standard therapy for hyperhomocysteinemia.

In conclusion, homocysteine is not linearly correlated with zinc, but is significantly associated by a cubical model, with a coefficient determinant of R^2=53.2%.

Conflict of Interest

None declared.

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