

## Selenium level in steroid-resistant and steroid-sensitive nephrotic syndrome

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### Abstract

**Background** The mechanisms of pathogenesis of steroid-resistant nephrotic syndrome (SRNS) and steroid-sensitive nephrotic syndrome (SSNS) are not well understood. Antioxidants, such as glutathione peroxidase enzyme (GPx) and its cofactor, selenium, are thought to slow the progress of nephrotic syndrome (NS).

**Objective** To compare selenium levels in SRNS and SSNS pediatric patients.

**Methods** This cross-sectional study was conducted in 51 SRNS and 30 SSNS patients, aged 2 to 18 years, who visited the Pediatric Nephrology Outpatient Clinic at Cipto Mangunkusumo Hospital, Jakarta. Subjects were included by consecutive sampling. Selenium level was measured on venous blood using GC tools MS™ (Agilent Technologies, inc.).

**Results** Median selenium levels in SRNS patients were 92 (range 42-154) µg/L and in SSNS patients were 93 (range 69-193) µg/L.

**Conclusion** Selenium levels in SRNS and SSNS patients were not significantly different. [Paediatr Indones. 2020;60:316-20; DOI: 10.14238/pi60.6.2020.316-20].

**Keywords:** children; selenium; antioxidant; nephrotic

**N**ephrotic syndrome (NS) is a clinical spectrum of the most common kidney disorders in children. It is characterized by massive proteinuria, hypoalbuminemia, edema, and hypercholesterolemia. Depending on the etiology, NS in children can be divided into congenital, primary, and secondary NS, with idiopathic NS being

the most common cause.<sup>1,2</sup> Depending on patient response to steroids, NS can also be categorized as steroid-sensitive or steroid-resistant.<sup>3,4</sup>

Idiopathic NS is associated to systemic T lymphocyte dysfunction, which releases capillary permeability factors, namely, toxic cytokines and free radicals.<sup>5</sup> Cytokines or free radicals interact with glomerular capillary walls through surface receptors. Intracellular changes then trigger an increase in the permeability of the glomerular basement membrane, causing plasma albumin to leak through the glomerular wall. Changes in the glomerular basement membrane load are due to reduced heparan sulfate proteoglycans and glomerular basal membrane sialic acid, which is caused by an increase in heparan sulfate catabolism in the glomerular basement membrane. The loss of the negative charge of the glomerular basement membrane result in a loss of rejection between the

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glomerular basement membrane and the negatively-charged plasma protein, leading to an increase in the permeability of the glomerular basement membrane wall, and hence, proteinuria.<sup>5-7</sup>

Aside from T lymphocyte dysfunction, free radicals also play a part in the pathogenesis of NS.<sup>5</sup> An imbalance of free radicals and antioxidants could cause oxidative stress, which occurs in NS. The effects of free radicals can be reduced with the use of antioxidants, such as zinc, boron, selenium, and vitamin C.<sup>8,9</sup> Selenium is a micronutrient that acts as a cofactor to glutathione peroxidase (GPx) and can function as an antioxidant.<sup>9,10</sup> A lack of selenium is suspected to influence the progress of NS that develops into SSNS or SRNS, because of an imbalance in antioxidants and free radicals. A study reported significantly decreased plasma selenium levels in active NS cases, with the lowest level detected during the initial attack. Selenium levels that returned to normal after remission is suggestive of interference with the defense mechanism caused by antioxidants.<sup>9</sup>

To date, little study has been done on selenium levels in NS patients based on response to steroid treatments. As such, we aimed to assess selenium levels in steroid-sensitive and -resistant NS based on their response to steroid treatments.

## Methods

The study was conducted using a cross-sectional method from November to December 2019 at Cipto Mangunkusumo Hospital, Jakarta. The subjects were SSNS and SRNS patients aged 2-18 years. Subjects were recruited consecutively until the minimum required number of subjects was met. Patients with nephrological abnormalities other than NS, such as congenital abnormalities involving the urinary system, urinary tract infections (UTI), malignancies, secondary NS, congenital NS, and infected patients were excluded. Sensitive-steroid NS patients were those in remission after receiving full doses of prednisone for 4 weeks, while SRNS patients were those not in remission after receiving full doses of prednisone (2 mg/kgBW/day) for 4 weeks.<sup>2</sup> In our study, some subjects were on steroidal and non-steroidal immunosuppressant therapy, while others received neither. Characteristics of subjects, including

age, sex, drug therapy, and serum creatinine levels, were obtained from medical records. Kidney function was assessed by glomerular filtration rate (GFR), using the Schwartz equation.<sup>11</sup> Venous blood (3.5 mL) was obtained from all subjects. Selenium examination of the blood was performed using GC tools MS™ (Agilent Technologies, inc.). The normal selenium level for patients older than 1-year was 23-190 µg/L based on Arup laboratories.<sup>12</sup> Mann-Whitney test was used for statistical analysis. This study was approved by the Ethics Committee of the University of Indonesia Faculty of Medicine and Cipto Mangunkusumo Hospital. Informed consent was provided by subjects' parents.

## Results

During the study period, 82 SSNS and SRNS patients met the inclusion criteria for this study. However, one patient did not have their blood drawn, resulting in a total of 81 subjects [51 (61.8%) with SRNS and 30 (38.2%) with SSNS]. The median ages were 7 years (SSNS) and 9.8 years (SRNS). There were more males than females in both groups (Table 1).

The mean GFR was 126 (SD 40.76) mL/minutes/1.73m<sup>2</sup> in the SSNS group and 102.94 (SD 41.98) mL/minutes/1.73m<sup>2</sup> in the SRNS group. Steroid therapy alone was given to 77.4% of the SSNS group and 24.0% of the SRNS group. Other immunosuppressant therapy was given to 6.4% of the SSNS group and 8.0% of the SRNS group. A combination of steroid and other immunosuppressant therapy was given to 6.4% of the SSNS group and 58.0% of the SRNS group (Table 1).

The median selenium levels were 93 (range 69-193) µg/L in the SSNS group and 92 (range 42-154) µg/L in the SRNS group. Statistical analysis revealed no significant difference in selenium levels between groups (P=0.583) (Table 2).

## Discussion

We assessed selenium levels in SSNS and SRNS patients. Selenium is an important element with various roles in the body. It is an exogenous antioxidant and a cofactor of the endogenous enzyme, glutathione

**Table 1.** Subjects' characteristics based on type of NS

Characteristics	SSNS (n=31)	SRNS (n=50)
Median age (range), years	7 (2-18)	9.75 (1.5-18)
Gender, n(%)		
Male	20	32 (64.0)
Female	11	18 (36.0)
Therapy		
Steroid alone	24	12 (24.0)
Non-steroidal immunosuppressant	2	4 (8.0)
Steroid and non-steroidal immunosuppressant	2	29 (58.0)
Received neither steroid nor non-steroidal immunosuppressants	3	5 (1.0)
Mean GFR (SD), mL/minutes/1.73m <sup>2</sup>	126 (40.76)	1 02.94 (41.98)

**Table 2.** Selenium levels in SSNS and SRNS patients

Variables	Group		P value
	SSNS (n=31)	SRNS (n=50)	
Median selenium (range), g/L	93 (69-193)	92 (42-154)	0.583

peroxidase (GPx) that changes H<sub>2</sub>O<sub>2</sub> substrates into H<sub>2</sub>O and O<sub>2</sub> through a redox reaction.<sup>13</sup>

The pathophysiology of NS is marked by the presence of free radicals, which leads to oxidative stress. Oxidative stress causes an increase in glomerular permeability, thus allowing plasma albumin to leak through the glomerulus wall.<sup>7</sup> Selenium and GPx play a role in maintaining cell membrane integrity and preventing damage caused by oxidative stress.<sup>10</sup> A study reported significantly decreased selenium in NS patients, with the lowest level during the initial attack, compared to frequent and infrequent NS relapse, and returning to normal levels during remission.<sup>9</sup> A previous study reported that selenium levels were low during the initial attack and remission compared to the healthy children, but selenium levels during remission were higher than during the initial attack.<sup>8</sup> This finding suggests that selenium might have a role in NS pathogenesis, especially during the initial attack.

In contrast to previous studies, we found no significant differences in selenium levels (P=0.58) between SSNS and SRNS subjects.<sup>8,9</sup> Variations in examination time, subject inclusion, research method, examination tools, and other exclusion criteria may have led to differences in the results.

Normal selenium levels might have been due to steroid use which can result in damage to mitochondria, the site of redox reactions.<sup>14-16</sup> A previous study

noted that steroids, namely, glucocorticoids, caused a decrease in mtRNA transcription and could impair the physiology of mitochondria. High doses of glucocorticoids for a long period result in excessive steroid influx through steroid receptors on the mitochondrial surface. This reduces mitochondrial ability to oxidize and decreases the mitochondrial membrane potential.<sup>16</sup> Disruption during redox could prevent GPx from converting substrate, such that selenium, which acts as a cofactor, would not be used in this reaction. Hence, normal or even high of levels of selenium can be detected in NS patients. Similarly, a study mentioned that normal selenium levels were detected in SSNS patients during the edema phase, at the end of steroid treatment, and remission, but were high at the end of high-dose steroid treatments.<sup>10</sup> In addition, another study noted an increase in selenium levels in patients with systemic lupus erythematosus, asthma or malignancies who received steroid therapy for 2 weeks.<sup>17</sup>

Three past studies reported that steroid therapy could cause interference during redox, resulting in normal selenium levels.<sup>14-16</sup> However, this statement has not been entirely proven. The normal selenium levels could have been influenced by factors other than steroid use. We confirmed this finding, as our subjects who did not receive steroid treatments also had normal selenium levels.

Some subjects in our study received non-

steroidal immunosuppressive therapy, using azathioprine, cyclophosphamide, calcineurin inhibitors, and mycophenolate mofetil. The use of other immunosuppressants might interfere with redox reactions in the body, as with steroid usage. However, this theory remains unverified.

The absence of a difference in selenium levels between the two NS classifications indicate that other factors besides selenium as an antioxidant influenced the differentiation of NS into steroid-resistant or steroid-sensitive. Genetic factors may induce SRNS. Mutations in more than 70 genes that encode podocyte proteins are known to trigger SRNS. In addition, other mutations may be involved, such as in genes that encode for foot process regulation of the actin network or in genes that encode the interaction of foot process with the basal glomerular membrane.<sup>18</sup> In addition to genetic factors, there is evidence that circulating factors can also cause SRNS, but it remains debatable.<sup>18,19</sup> Previous studies stated that zinc, as an antioxidant, has a role in reducing the frequency of relapse in SSNS patients.<sup>20,21</sup> As such, other antioxidants might have a role in NS differentiation into SSNS or SRNS. Furthermore, anatomical pathology has a role in the differentiation of NS into SRNS or SSNS. In children, the majority of idiopathic NS (80%) patients have the anatomic pathology of minimal change disease (MCD). After the initial corticosteroid treatment, most MCD patients (94%) experienced total remission, whereas, in focal segmental glomerulosclerosis patients (FSGS), 80-85% of them become steroid-resistant.<sup>2</sup>

A limitation of this study was that selenium examinations were carried out on patients who had received steroid therapy and non-steroidal immunosuppressants, since the study took place at a national referral center. As a result, most patients received steroid therapy and non-steroidal immunosuppressants before the study. Another limitation was the use of American laboratory standards for selenium levels, as Indonesian standards do not exist. The last limitation is not comparing selenium levels with normal healthy controls. In conclusion, there is no significant difference in selenium levels in pediatric SSNS and SRNS. Further study is needed using case-control studies, and selenium examination must be carried out serially before NS patients receive steroid therapy.

## Conflict of Interest

None declared.

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