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Vascular Endothelial Growth Factor (VEGF) and Neopterin Levels in Children with Steroid-sensitive and Steroid-resistant Nephrotic Syndrome

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ABSTRACT

Background: The most common glomerular disease in children is nephrotic syndrome. Steroid-resistant nephrotic syndrome tends to have a worse disease course, which bears a significant risk of chronic kidney disease in children. **Objective:** To compare VEGF and neopterin levels between children with steroid-sensitive nephrotic syndrome (SSNS), steroid-resistant nephrotic syndrome (SRNS), and also healthy (control) children. **Methods:** This cross-sectional study was conducted at H. Adam Malik General Hospital, Indonesia from January to December 2018. There were 160 children aged 1 to 8 years with confirmed nephrotic syndrome and without end-stage renal disease and systemic diseases, divided into SSNS, SRNS, and control groups. Data regarding age, gender, urine albumin creatinine ratio (UACR), serum albumin, total cholesterol, urea, creatinine, VEGF, and neopterin levels were collected. A p-value of less than 0.05 is considered statistically significant. **Results:** There were no differences between groups in gender ($p = 0.269$) and age ($p = 0.375$), but there was significant difference of UACR, albumin level, total cholesterol level, and VEGF level between groups, (all $p< 0.001$). There was a moderate positive correlation between VEGF level and UACR ($r(158) = 0.439$, $p< 0.001$) and a moderate negative correlation between neopterin level and albumin level ($r(158)= -0.312$, $p = 0.005$). **Conclusion:** There were no differences in serum VEGF and neopterin levels between steroid-sensitive and steroid-resistant nephrotic syndrome groups. Serum VEGF level was positively correlated with UACR while serum neopterin level was negatively correlated with serum albumin level.

Keywords: child, neopterin, nephrotic syndrome, vascular endothelial growth factors.

1. BACKGROUND

The most common glomerular disease in children is nephrotic syndrome (1-3). Its global prevalence ranged from 1,15 to 16,9 per 100.000 children (4). In Indonesia, the prevalence of nephrotic syndrome in children is 6 out of 100 000 population. Males are more dominant compared to females at a ratio of 1.5 to 2:1 (5).

Nephrotic syndrome is a group of disorders consists of proteinuria (>40 mg/m2/hour or urine protein/creatinine ratio ≥200 mg/mL or 3+ protein on urine dipstick), hypoalbuminaemia (<2.5 g/dL), oedema, and hypercholesterolaemia (>200 mg/dL) (5). It is chronic and frequently relapsed (2). Based on histology, there are several types of nephrotic syndrome, including minimal change nephropathy and focal segmental glomerulosclerosis. The first one is commonly steroid-sensitive while the latter is steroid-resistant (3). Steroid-sensitive nephrotic syndrome is defined as complete disease remission in 4 weeks as a response to full dose steroid therapy (7), otherwise categorized as steroid-resistant (8). Steroid-resistant nephrotic syndrome tends to have a worse disease course (6). It bears a significant risk of chronic kidney disease in children and rapid progression to renal failure, which affects the physical and psychological aspects of children in future years. Impaired quality of life in children suffering nephrotic syndrome, worsened on children with steroid resistance (9).

Hospitalizations and relapses are also associated with a higher economic burden. Between 2006 and 2009, the economic burden from medical-related costs for nephrotic syndrome in the United States of America was reported at

259 million USD (10). Therefore, differentiating between steroid-sensitive and steroid-resistant nephrotic syndrome is crucial in accelerating diagnosis, maximizing patients' outcomes, and cost-effective treatment (11). Performing renal biopsy and histological examination, as the gold standard to obtain a diagnosis, is highly invasive and post-biopsy complications are inevitable (12). Serum and urinary biomarkers could be used to differentiate both entities (3). Vascular endothelial growth factor (VEGF) and neopterin are some biomarkers being evaluated recently (3, 13). VEGF is an endothelial growth factor, which is important in endothelial cell differentiation and angiogenesis (14). VEGF plays important role in increasing vascular permeability (13, 15), which aggravates proteinuria in nephrotic syndrome. In steroid-sensitive cases, steroid administration reduces serum and urine VEGF levels (14).

Neopterin is a pyrazine-pyrimidine compound produced by monocytes and macrophages following the inflammatory process (15, 16). It enhances macrophage cytotoxicity (16). Neopterin is a representation of cell-mediated immunity (16). Serum and urine neopterin level was associated with inflammation and tubular damage in children with chronic kidney disease. High neopterin level indicates a more progressive kidney disease course (15).

2. OBJECTIVE

This study aimed to compare VEGF and neopterin levels between children with steroid-sensitive and steroid-resistant nephrotic syndrome and also healthy children as controls.

3. MATERIALS AND METHODS

3.1. Participants and ethical considerations

This cross-sectional study was conducted at H. Adam Malik General Hospital Medan, North Sumatera, Indonesia from January to December 2018. The diagnosis of nephrotic syndrome was based on the standard clinical picture: massive proteinuria (more than 40 mg/m2/hour or urinary protein/creatinine ratio (PrU/CrU) in urine >2 mg/mg or urine dipstick ^{3}2+), hypoalbuminemia £2.5 g/dL, and edema or with nephrotic syndrome in complete remission confirmed by negative or trace proteinuria for three consecutive days. Subjects whose clinical picture meets the criteria were further divided into the SSNS group (achieving complete remission within the initial 4 weeks of steroid therapy) and SRNS group (failed to achieve complete remission after 8 weeks of corticosteroid therapy). Renal biopsy was not performed in patients with idiopathic nephrotic syndrome in this study, as it fits the standard clinical picture of minimal change nephrotic syndrome (14). Children with end-stage renal disease (GFR≤ 60 mL/minute/1.73m2 body surface area) and systemic diseases (malignancy, pulmonary tuberculosis, severe malnutrition, obesity, heart disease, liver abnormality, SLE, and Henoch-Schönlein purpura) were excluded.

A total of 160 children aged 1 to 8 years were enrolled in this study, which comprised of 80 children with nephrotic syndrome (further divided into SSNS and SRNS groups) and control (80 healthy children) groups after approved by the Institutional Review Board of Universitas Sumatera Utara (No.419/TGL/KEPK FK USU-RSUP HAM/2017).

3.2. Measures

Data including age, gender, urine albumin-to-creatinine ratio (UACR), serum albumin level, total cholesterol level, urea level, creatinine level, VEGF level, and neopterin level was collected from each child's medical record. Urine samples were obtained from spot urine, and 15 ml peripher blood was drawn.

VEGF levels were determined using Quantikine ELISA kit human VEGF (R&D Systems, Inc., Minneapolis, USA). A total of 50 µL of standards and 10 µL of samples' sera were added to the wells filled with 100 µL of assay diluent. The plate was sealed before 2 hours incubation on a microplate shaker at room temperature. The wells were washed for 4 times in total before 200 µL of conjugate were added. A different plate seal was used before underwent 2 hours incubation and washed with the mentioned technique above. The substrate solution was added to each well with a volume of 200 µL. Protected from light, the plate underwent 30 minutes incubation at room temperature. Finally, 50 µL of stop solution was added before VEGF concentration was measured using optical densitometry at the wavelength of 450 nm (17).

Neopterin levels were measured using neopterin enzyme immunoassay 96-kit (IBL GmbH, Hamburg, Germany). 20 µL of standards and serum was added to the wells, later added with 100 µL of enzyme conjugate and 50 µL of neopterin antiserum. The plate was later covered with black adhesive foil and underwent 90-minutes incubation at room temperature on an orbital shaker in the dark. Afterward, wells were washed 4 times and tapped on a paper towel to dry out excess liquid. TMB Substrate solution (150 µL) and stop solution (150 µL) were mixed, proceeded with optical density examination to measure serum neopterin level (18). All laboratory parameters were obtained when administered to the hospital.

3.3. Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (IBM Corporation, New York, USA). Chi-square test was used for comparison between categorical variables and Fisher's exact test would be used if Chi-square test assumptions were not met. One-way ANOVA test followed by posthoc Bonferroni test was used for numerical variables between control, steroid-sensitive nephrotic syndrome (SSNS), and steroid-resistant nephrotic syndrome (SRNS) groups in normal data distribution, otherwise Kruskal-Wallis test followed by serial Mann-Whitney U test would be used. Correlations between VEGF and neopterin and UACR, albumin, total cholesterol, urea, and creatinine levels were determined using the Pearson correlation test in the normal distribution, otherwise, the Spearman correlation test was used. P-value less than 0.05 is considered statistically significant.

Variables	SRNS	SSNS	Control	p
Gender				
Male	36 (64.3%)	19 (79.2%)	49 (61.25%)	0.269
Female	20 (35.7%)	5 (20.8%)	31 (38.75%)	
Age, years	5 (2 – 8)	4 (2 – 7)	4 (2- 8)	0.357
UACR, mg/g	3.1 (2.6 – 5.2) #	3 (2.3 – 5)#	0.3 (0.2 – 1.3)	<0.001*
Albumin, g/dL	2 (1 – 2.4) #	2 (1 – 2.5)#	4 (3.5 – 4.8)	<0.001*
Total cholesterol, mg/dL	300 (140 – 400)#	287 (120 – 400)#	160 (120 – 190)	<0.001*
Urea, g/dL	19 (13 – 48)	19 (13 – 48)	19 (13 – 45)	0.830
Creatinine, g/dL	0.7 (0.45 – 1.1)	0.8 (0.6 – 1.2)	0.7 (0.4 – 0.86)	0.09
VEGF, pg/dL	517 (264.9 – 880.4) #	391.1 (145.5 – 963.4) #	156.8 (40.3 – 454.1)	<0.001*
Neopterin, nmol/L	34.9 (16.23 – 72.01) #	24.3 (0.45 – 81.34) #	6.17 (0.24 – 30.41)	<0.001*

Table 1. Demographic and laboratory characteristics of the patients. *p<0.05; # significant than control; SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome; UACR, urine albumin creatinine ratio; VEGF, vascular endothelial growth factor

	VEGF		Neopterin	
	p	r	P	r
UACR	<0.001*	0.439	0.788	0.030
Albumin	0.244	-0.132	0.005*	-0.312
Total cholesterol	0.609	0.058	0.051	0.219
Urea	0.664	0.049	0.154	0.161
Creatinine	0.140	-0.167	0.817	-0.026

Table 2. Correlation between vascular endothelial growth factor (VEGF) and neopterin and urine albumin creatinine ratio (UACR), total cholesterol, urea, and creatinine. *p<0.05; VEGF, vascular endothelial growth factor; UACR, urine albumin creatinin ratio

4. RESULTS

A total of 160 children were enrolled in this study, comprised of 56 children on the SRNS group, 24 children on the SSNS group, and 80 children on the control group. There were no differences between groups in gender (p = 0.269) and age (p = 0.375) UACR, Albumin, total cholesterol, VEGF and Neopterin levels were found significantly different between control group compared to SSNS and SRNS group (p<0.001). But, serum urea and creatinine level were found not different significantly in between groups (p = 0.830; p = 0.09, respectively) (Table 1).

There was a moderate positive correlation between VEGF level and UACR (r(158) = 0.439, p<0.001) and a moderate negative correlation between neopterin level and albumin level (r(158)= -0.312, p = 0.005) (Table 2).

5. DISCUSSION

Nephrotic syndrome is a common chronic disease in children, mainly affects children aged two to six years (2, 6). It is concordant with our findings in study subjects. Less than 10% of children with nephrotic syndrome develop resistance toward steroids and need alternative treatments (2, 3).

Children with steroid-resistant nephrotic syndrome have a higher risk of progression toward end-stage renal disease (9). The findings of this study showed lower albumin levels and higher total cholesterol levels in nephrotic syndrome patients compared to controls, which were concordant to the pathophysiology of the disease (19). This might be related to the arising hypothesis found in previous studies, which stated that there was existed one or more circulating factors that influence the permeability of renal vascular in nephrotic syndrome (13, 15).

Farid et al. found VEGF level was found higher in patients with nephrotic syndrome compared to normal subjects (15). Ostalska-Nowicka, et al. found that the VEGF-C level was increased in children with steroid-resistant nephrotic syndrome and focal segmental glomerulosclerosis (FSGS) compared to control and minimal change disease, based on histologic examination (20). All results of the above studies were concordant to the findings in our study, serum VEGF level was found to be higher in nephrotic syndrome groups compared to the control group. In experimental study using mice, Veron et al. explained the overexpression of VEGF caused albuminuria and microscopic changes in glomerulus resembling nephrotic syndrome and became not responsive towards steroid administration, which indicates to steroid resistance (21).

In contrast, Farid et al. did not find any difference in VEGF levels based on steroid sensitivity in nephrotic syndrome groups, with no significant correlation found between serum VEGF level and UACR in the study (15). In concordance to this study, there was no difference shown in serum VEGF level between SSNS and SRNS groups.

This study found that there was a positive moderate-to-low correlation between serum VEGF level and UACR. It is concordant with a study by Kim et al. in which UACR was found to be weak positively correlated with plasma (r(162) = 0.251, p = 0.044), and moderate strength of correlation with urinary VEGF (r(162) = 0.645, p < 0.001) (22). Webb et al. showed discordance to previous studies' results, as it reported that the VEGF level was not significantly different between relapse and remission groups and also with a healthy control group (13). In Ataga et al. found significant negative correlation between UACR and VEGF level (r(21) = -0.49, p = 0.015). The negative correlation between UACR and VEGF was explained as podocytes in the glomerular basement membrane generate and respond to angiogenic growth factors, including VEGF. Damage in VEGF signaling or maturity would cause endothelial damage (23), which affects UACR. Renal endothelial damage occurs in glomerular diseases but was not thought to occur

in common pathophysiology of nephrotic syndrome in children, which mostly were idiopathic cases (1)

This study found that neopterin levels were higher in active nephrotic syndrome compared to controls. A positive correlation was also observed between neopterin and the degree of proteinuria in those children, which was concordant with the finding by a study from Bakr, et al. (24) and Oda et al. (17). Yadav et al. also showed that serum neopterin level was also associated with the severity of chronic kidney disease (25).

In active cases, it is also important to analyze further between SSNS and SRNS cases. However, our study findings showed that serum neopterin level was elevated in children with nephrotic syndrome compared to healthy children, but not significantly different between children with SSNS and SRNS. This is concordant with a study Uwaezuoke et al (3) which found no difference in neopterin levels between steroid-sensitive and steroid-resistant nephrotic syndrome. Neopterin, one of the markers of disease activity was useful in diagnosing active nephrotic syndrome as its level reflects T-cells function (25). However, neopterin was unable to differentiate steroid-resistant from steroid-sensitive patients, nor to predict prognoses (16). It is suggested from a study by Oda et al, that the steroid therapy (which primarily classified patients into SSNS and SRNS) may decrease the increasing production of neopterin which supposedly occurred, by normalizing disturbed T-cell function (17).

In this study, serum neopterin level was found to be negatively associated with serum albumin level. This is concordant to a previous study by Lhee HY et al. (26). A negative correlation might be explained as albumin is one of the proteins whose level decreases upon progressivity of the inflammation process (27), in which the neopterin level would rise.

This study has several limitations. Other confounding factors affecting serum VEGF and neopterin levels, such as polymorphism, were within the limitation of this study. A larger study involving more subjects and several centers is needed to confirm the findings of this study.

6. CONCLUSION

Serum VEGF and neopterin levels were higher in children with nephrotic syndrome compared to healthy children. There were no differences in serum VEGF and neopterin levels between steroid-sensitive and steroid-resistant nephrotic syndrome groups. Serum VEGF level was positively correlated with UACR while serum neopterin level was negatively correlated with serum albumin level.

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