Research Article

Serum Nephrin Levels in Severe Preeclampsia: A Cross-Sectional Study

Kadar Nephrin Serum pada Preeklamsia Berat: Sebuah Studi Potong Lintang

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Abstract

Objective: To determine differences in serum nephrin levels in severe preeclampsia compared to normal pregnancy and also its correlation with systolic and diastolic blood pressure and proteinuria.

Methods: This study is an analytical observational with cross sectional study. The observation group consisted of severe preeclampsia (n= 30) and normal pregnancy group as a control (n= 30). Both groups measured systolic and diastolic blood pressure, proteinuria and serum nephrin using the Human NPHN (Nephrin) ELISA Kit. Statistical test were performed with Mann-Whitney test and the Spearman's rank test. A value of $p<0.05$ was considered significant. The study was conducted in the Obstetric Clinic Inward and Laboratorium Department of Clinical Pathology Dr. Hasan Sadikin General Hospital/Faculty of Medicine Universitas Padjadjaran on March–May 2019.

Results: Levels of serum nephrin in the severe preeclampsia group were significantly higher than in normal pregnancies (6.4 ng/mL vs 4.2 ng/mL; p= 0.014). There is a positive weak correlation but statistically significant between serum nephrin with systolic blood pressure (r= 0.36; p= 0.02) but not significant to diastolic blood pressure (r= 0.3; p= 0.05). There is no significant correlation was found between serum nephrin levels and proteinuria (r= 0.18; p= 0.54).

Conclusions: Levels of serum nephrin in the severe preeclampsia group were significantly higher than in normal pregnancies and there is a correlation between serum nephrin with systolic blood pressure.

Keywords: blood pressure, proteinuria, serum nephrin, podocyte, severe preeclampsia.

Abstrak

Tujuan: *Untuk mengetahui perbedaan kadar nephrin serum pada preeklamsia berat dibandingkan dengan kehamilan normal dan juga hubungannya dengan tekanan darah sistolik dan diastolik serta proteinuria.*

Metode: *Penelitian ini bersifat analitik observasional dengan pendekatan potong silang. Kelompok pengamatan terdiri dari kelompok preeklamsia berat (n= 30) dan kelompok kehamilan normal sebagai kontrol (n= 30). Pada kedua kelompok dilakukan pengukuran tekanan darah sistolik dan diastolik, proteinuria serta pengukuran kadar nephrin serum menggunakan Human NPHN (Nephrin) ELISA Kit. Uji statistik dilakukan dengan uji Mann-Whitney dan uji rank Spearman. Nilai p<0,05 dianggap bermakna. Penelitian dilakukan di ruang perawatan obstetri FKUP/RSHS dan Laboratorium Patologi Klinik FKUP/RSHS pada bulan Maret-Mei 2019.*

Hasil: *Rerata kadar nephrin serum pada kelompok preeklamsia berat lebih tinggi secara bermakna dibandingkan kehamilan normal (6,4 ng/mL vs 4,2 ng/mL; p= 0,014). Terdapat korelasi positif dengan derajat lemah namun bermakna secara statistik antara nephrin serum dengan tekanan darah sistolik (r= 0,36; p= 0,02) namun tidak signifikan terhadap tekanan darah diastolik (r= 0,3; p= 0,05). Tidak ditemukan korelasi yang bermakna antara kadar nephrin serum dengan proteinuria (r= 0,18; p= 0,54).*

Kesimpulan: *Kadar nephrin serum pada kelompok preeklamsia berat lebih tinggi dibandingkan kehamilan normal dan terdapat korelasi antara nephrin serum dengan tekanan darah sistolik.*

Kata kunci: *nephrin serum, podosit, preeklamsia berat, proteinuria, tekanan darah.*

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INTRODUCTION

Severe preeclampsia (SP) is a major cause of maternal and perinatal morbidity and mortality. In the world, severe preeclampsia occurs around 5-8%.[1-3] SP according to ACOG is if any of the following symptoms are found: increased systolic ≥ 160 mmHg, diastolic ≥ 110 mmHg; thrombocytopenia (<100,000 / mL); elevated liver enzymes (> 2 times), right upper abdominal pain or epigastric pain; serum creatinine> 1.1 mg/dL; pulmo edema; cerebral or visual impairment. Proteinuria in SP if the levels was 300 mg or more which obtained with urine 24 hours or +1 by dipstick, but proteinuria is not an examination to diagnose SP. [4] The incidence of SP and the complications is still quite high. This is because of the pathophysiology of preeclampsia is still unclear although many studies on the pathophysiology of preeclampsia have been carried out, including endothelial damage. [2, 5, 6] Other pathophysiology was known is angiogenic imbalance, sFlt-1 and sEng which causes endothelial and podocyte damage and causing proteinuria. Vascular endothelial growth factor (VEGF) blockers are known to induce endothelin-1 (ET-1) causing podocyte damage. 2, [7-9] Vasoconstriction also causes neurohormonal system disorders including activation of the renin angiotensin aldosterone system (RAAS) known as ET-1. [2, 10, 11] RAAS plays an important role in the regulation of BP and electrolyte balance as well as work on the kidney system.

Endothelial damage causes vasoconstriction and hypoxia resulting in systemic organ malfunction, one of it is in the kidneys. [2, 12-14] Glomerular endotheliosis occurs in the kidney with thrombotic microangiopathy characterized by endothelial glomerular swelling and capillary lumen occlusion so that glomerular volume increases with severity and there is proteinuria which can cause chronic kidney failure or end stage renal disease (ESRD). [1, 12, 15] This happened in glomerular podocytes consisting of podocin, synaptopodin, podocalyxin and nephrin which play a role in maintaining the integrity of the glomerular barrier protein slit diaphragm.[2, 12, 16, 17]

Nephrin can be damaged in certain disease, including congenital nephrotic syndrome of the Finnish type (NPHS1), minimal change disease (MCD), membranous glomerulopathy, focal segmental glomerulosclerosis (FSGS), and nephropathic arthritis. [12, 18-20] Nephrinuria is the result of glomerular slit diaphragm damage that occurs in SP, related to the severity of proteinuria, also correlates with serum creatinine, illustrating renal function in SP.

Dipstick proteinuria is a semiquantitative examination which is a rough estimate to assess urine concentration, influenced by the amount of urine produced when taking urine samples. This can cause false negatives or false positives. Serum nephrin levels are known to be higher than urine nephrin, that the protein composition in the urine is different from the plasma protein composition. Plasma protein contains ± 60% urine protein, while urine protein is only about 20%.[21] The difference is influenced by gestational age, the more gestational age so the serum nephrin levels will decrease. Nephrin can be detected before proteinuria occurs and the clinical appearance of severe preeclampsia appears, therefore nephrin is used as a marker of subclinical kidney damage. [3, 12, 18-20, 22]

METHODS

This research is an analytical observational with cross sectional study. The observation group consisted of SP (n= 30) and normal pregnancy group as a control (n= 30). Both groups measured systolic and diastolic BP, proteinuria and serum nephrin using the Human NPHN (Nephrin) ELISA Kit. Research subjects were taken used consecutive sampling to inclusion criteria. Statistical test were performed with Mann-Whitney and the Spearman's rank test. A value of $p<0.05$ was considered significant. The study was conducted in the Obstetric Clinic Inward and Laboratorium Department of Clinical Pathology Dr. Hasan Sadikin General Hospital/ Faculty of Medicine Universitas Padjadjaran on March–May 2019. Analysis and data processing carried out by the researcher and statistic supervisor. This is done manually and computerized by using the software program Statistical Product and Service Solution (SPSS) for Windows version 25.0.

RESULTS

After conducting a study using a cross sectional study with consecutive sampling in each of the 30 patients in the severe preeclampsia group and the normal group with gestational ages over 20 weeks to 34 weeks who met the inclusion criteria in the Obstetrics and Gynecology Section of the Faculty of Medicine Unpad/Hasan Sadikin Hospital Bandung. The characteristics of the subjects are presented in Table 1.

Table 1. Characteristics of the Subjects

Characteristic	Group		*P-value*
	SP (n=30)	SP (n=30)	
Age(years old)			
<24	9 (30)	7(23)	0.788
25-29	19 (63)	20 (67)	
30-34	2 (7)	3 (10)	
Average (SD)	2.7 (3.6)	25.8 (2.9)	
Range	20-34	20-30	
Gestational age (week)			
20-28	2 (7)	2 (7)	1.0
29-34	28 (93)	28 (93)	
Nutritional status (BMI)			
Normal	1 (3)	11 (37)	0.002
Overweight	1 (3)	3 (10)	
Obesity	28 (94)	16(53)	
Average (SD)	30.6 (4.8)	25.4 (3.7)	
Range	21.6-44.4	20.3-32.4	

**) chi-square*

Table 2. Blood Pressure Research Subjects

Characteristic	Group	
	SP (n=30)	SP (n=30)
Systolic (mmHg)		
Average (SD)	170.3 (15.0)	109.3 (10.8)
Range	160 – 230	80 – 120
Diastolic (mmHg)		
Average (SD)	106.0 (8.9)	72.7 (6.4)
Range	100 – 130	60 – 80

Table 3. Comparison of Serum Nephrin Levels in Severe Preeclampsia and Normal Pregnancy

Serum Nephrin (ng/mL)	Group		*P-value*
	SP (n=30)	SP (n=30)	
Average (SD)	6.4 (3.64)	4.2 (1.89)	0.014
Median	5.4	4.1	
Range	1.7 – 14.8	0.8 – 7.5	

**) Mann-Whitney test*

Table 4. Correlation of Nephrin Serum Rates with Proteins and Blood Pressure

Correlation Nephrin Serum with	SP		Normal	
	r	*P-value*	r	*P-value*
Proteinuria 24 hours	-0.05	0.41	-	-
Dipstick	0.18	0.54	0.68	0.01
Systolic	0.36	0.02	0.02	0.91
Diastolic	0.30	0.05	0.23	0.22

r= Spearman's rank correlation coefficient

DISCUSSION

Table 1 presents data on the characteristics of pregnancy with SP and normal pregnancy, it shows that the maternal age and gestational age of the two groups did not show a significant difference (p> 0.05). The most SP occurred in maternal age 25-29 years as 19 patients (63%) and 29-34 weeks gestation (early onset) as 28 cases (93%). In this study, the calculation of BMI by obtaining the patient's weight during examination (most of them did not know their weight before pregnancy) so that nutritional status was obesity as 28 patients (94%) in SP group and 16 patients (53%) in normal group. In table 2 shows the systolic of the SP group was 170.3 mmHg with a range of 160-230 mmHg and the diastolic was averaged 100-130 mmHg with range of 106.0 mmHg.

Based on table 3 Comparison of Serum Nephrin Levels in SP and Normal Pregnancy, the results showed in SP mean serum nephrin levels are 6.4 ng/mL and in normal pregnancies 4.2 ng/ mL. In the normal group the serum nephrin levels were 0.895 ng/mL and in the SP group the serum nephrin levels were 4.285 ng/mL with p values <0.001 (significant). It's also found significant differences in serum nephrin levels in severe preeclampsia (7.1 ng/mL) compared to the normal group (3.9 ng/mL).[3, 12] So it concluded that serum nephrin levels which is higher in the SP group than normal group.

From table 4, the relationship between nephrin levels with 24-hours proteinuria levels in SP showed the r = -0.05 with p = 0.41 (p> 0.05) which was not significant. The result was same with examination using protein dipstick, p> 0.05 which means not significant (r = 0.18 and p = 0.54).

This might be caused that proteinuria in SP is transient. Dipstick proteinuria is also a semiquantitative examination which is a rough estimate for assessing urine concentration, influenced by the amount of urine produced when taking urine samples so that the accuracy of urine collection must also be considered. Nephrinuria levels do not depend on serum nephrin levels so it is proven that nephrinuria concentrations do not originate from the systemic circulation.[12] As evidenced by Son et al, serum nephrin is found to be five times higher than urine. In addition it is also known that the composition of protein in the urine is different from the composition of plasma proteins. Plasma protein contains ±

60% protein while urine protein is only about 20%. This is in also conducted by Jung et al, found that serum nephrin levels increased at 21-28 weeks gestational age with serum nephrin levels 145.64 ng/mL compared to 29-40 weeks gestation (100.60 ng/mL), whereas for urinary nephrin levels at 21-28 weeks gestation urinary nephrin levels are 0.23 ng/mL and at 29-40 weeks gestation with urinary nephrin levels 2.11 ng/mL. So it can be concluded that at SP serum nephrin levels decrease along with increases gestational age and will increase during postpartum. This is contrary to urinary nephrin levels, increase along the gestational age and decreases during postpartum. [21, 23]

The relationship between serum nephrin levels with BP, obtained a weak and significant correlation with systolic with r = 0.36 and p = 0.02 (p <0.05) whereas at diastolic obtained a weak correlation with r = 0.30 but not significant with p = 0.05 (p> 0.05). In this study, isolated systolic hypertension may occur so that a significant correlation exists only in systolic blood pressure. This occurs at a young age with risk factors for obesity and occurs in peripheral blood vessels rather than central. The pathophysiologic mechanisms include the involvement of aging factors, increased arterial stiffness, increased endothelial damage, elastin calcification, increased sympathetic activity, and increased RAAS activity. Age, atherosclerosis progression, and arterial elastin build up will increase deposits of calcium and arterial collagen. Decreased elastic arteries and this ability to adjust will reduce the ratio of lumen to artery walls and increase arterial stiffness. This change mainly occurs in large arteries and aorta. Other studies have also shown that an increase in systolic blood pressure induces inflammation that causes endothelial dysfunction, vasoconstriction and thickening of the intima tunica and arterial media. Stiffness in the left ventricle and hypertrophy occur due to efforts to maintain a balanced cardiac output due to increased afterload. The results of ventricular remodeling reduce filling during diastolic and disturb diastolic relaxation. This stiffness causes an increase in systolic blood pressure and a decrease in diastolic. [24]

CONCLUSION

Levels of serum nephrin in the severe preeclampsia group were significantly higher than in normal pregnancies and there is a positive weak correlation but statistically significant between serum nephrin with systolic blood pressure but not significant to diastolic blood pressure. There is no significant correlation was found between serum nephrin levels and proteinuria.

SUGGESTION

Further research needs to be done on nephrinuria and proteinuria or serum nephrin with serum creatinine and the grouping of gestational age is not limited to early onset preeclampsia but also on late onset preeclampsia and also grouped if there are other complications such as Hellp Syndrome and others.

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