

## Correlation of serum albumin level with platelet count and aggregation in children with nephrotic syndrome

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### Abstract

**Background** Nephrotic syndrome (NS) is the most common renal disease among children, and contributes to life-threatening complications such as thromboembolic disease. Platelets are considered to be important agents in thrombotic events among NS patients. The gold standard assessment for platelet aggregation is the use of adenosine diphosphate (ADP) agonist, however, it is expensive and not accessible in smaller health facilities. Thus, other thrombosis parameters are needed. Previous studies suggested that low albumin increased the risk of thrombosis in NS patients.

**Objective** To assess for a possible correlation between albumin level and platelet count as well as platelet aggregation.

**Methods** This cross-sectional study was conducted in children with nephrotic syndrome who were admitted to the Pediatric Nephrology Department in Dr. Hasan Sadikin General Hospital, Bandung, West Java, from November 2017 to March 2018. Subjects were selected by consecutive sampling. Serum albumin, platelet count, and platelet aggregation were measured. Statistical analysis was conducted by Spearman's test.

**Results** A total of 32 patients participated in the study, with mean age of 109 (SD 7.4) months. Most subjects were male (56%). Subjects' mean serum albumin level was 2.06 (SD 1.23) g/dL; mean platelet count was 453,062.5 (SD 187,443.90)/mm<sup>3</sup>; and mean platelet aggregation values for ADP agonist concentrations of 10, 5, 2.5, and 1  $\mu$ M were 86.8 (SD 8.63)%, 82.4 (SD 15.33)%, 66.6 (SD 24.90)%, 34.95 (SD 31.69)%, respectively. Partial correlation analysis revealed significant negative associations between albumin and platelet count as well as platelet aggregation assessed with 1  $\mu$ M of ADP concentration ( $P < 0.001$ ), with Spearman correlation coefficients of -0.641 and -0.634, respectively.

**Conclusion** Serum albumin level has a moderately negative correlation with platelet count and platelet aggregation value. [Paediatr Indones. 2019;59:7-12; doi: <http://dx.doi.org/10.14238/pi59.1.2019.7-12> ].

**Keywords:** albumin; nephrotic syndrome; platelet aggregation value; platelet count

Nephrotic syndrome (NS) is the most common renal disease among children. A UK and US study stated the NS incidence as 2-7 new cases per 100,000 children annually, with prevalence around 12-16 cases per 100,000 children.<sup>1</sup> In Indonesia, new cases of NS among children below 14 years of age were about 6 per 100,000 children annually in 1992.<sup>2</sup> Nephrotic syndrome may contribute to life-threatening complications during its course, including thromboembolic disease, such as deep vein thrombosis with or without pulmonary emboli, infection, as well as iatrogenic complications such as immunosuppression or bone density loss due to long-term use of steroids, and acute kidney injury which may lead to chronic kidney disease.<sup>1,3</sup>

Thromboembolic events are rare but generally fatal for NS patients.<sup>4,5</sup> The risk of thrombosis in veins and arteries is caused by increased platelet count, platelet hyperaggregation, release of active substances, elevated platelet surface mediator

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Submitted November 22, 2018. Accepted February 5, 2019.

expression, and loss of low molecular weight proteins. Platelet are considered as an important agent of thrombosis among NS patients.<sup>6</sup> The gold standard for platelet aggregation requires the use of adenosine diphosphate (ADP) agonists, which are dense granule degranulation products that directly induce platelet aggregation, unlike thrombin and collagen which only trigger ADP and TXA2 release.<sup>7</sup> However, this examination may be affected by various medications such as NSAIDs, platelet receptor antagonists (*Abciximab*, *Clopidogrel*), glycoprotein IIb/IIIa inhibitor (*Eptifibatide*), phosphodiesterase inhibitor (*Dipyridamol*), anticoagulants (heparin, warfarin), diuretics, and vasodilators.<sup>8</sup> The ADP agonist test also may require a larger blood specimen (450  $\mu$ L of blood), can only be conducted in major laboratories, has a high predicted cost, and requires a long time to process the specimen.<sup>9</sup> Other modalities with lower cost, higher availability, and easier procedures are needed to predict thrombosis events.

During relapse, serum albumin level decreases due to urinary excretion. Recent studies showed that low albumin level may elevate the venous thromboembolic rate by 2 to 5.8 times.<sup>10</sup> Regarding the previous studies about the similar topic, this is the first study to assess for possible correlations between serum albumin level and other thrombosis parameters, such as platelet count and platelet aggregation value in pediatric NS patients.

## Methods

This cross-sectional study was conducted in the Pediatric Inpatient Ward and Pediatric Outpatient Clinic, Dr. Hasan Sadikin General Hospital, Bandung, West Java, between November 2017 – March 2018. This study was approved by the Health Studies Ethics Committee at Dr. Hasan Sadikin General Hospital, Bandung.

Subjects were selected by consecutive sampling. Children diagnosed with NS and whose parents provided written informed consent were included in this study. Patients with previous thrombotic events or hepatic function disorders were excluded. Patients who underwent treatment with thrombolysis, fibrinolysis, or NSAIDs were also excluded.

Serum albumin level was measured by a UV-VIS

*Spectrophotometer*, while platelet count was assessed by *Coulter* counter. Platelet aggregation was measured as the amount of ADP agonist needed to induce aggregation, using the Helena AggRAM test kit and software.

Data processing and analysis was done with SPSS 24.0 for Windows. Collected data were noted on the research form, edited, and coded prior to data analysis. Spearman's test was used to analyze for correlations between serum albumin level and platelet count, as well as platelet aggregation. Results with P values <0.05 were considered to be statistically significant.

## Results

A total of 32 patients participated in this study, with an age range of 17 to 210 months. Demographic characteristics are shown in **Table 1**. Most subjects were male and the mean patient age was 109 months (9 years and 1 month). Most patients were diagnosed as having resistant NS (21 subjects), followed by frequently-relapsing NS (6 subjects).

**Table 1.** Demographic characteristics of subjects

Characteristics	(N=32)
Sex, n	
Male	18
Female	14
Age, months	
Median (range)	102.5 (17-210)
Mean (SD)	109 (57.4)
Diagnosis, n	
First episode of NS	3
Frequently-relapsing NS	6
Steroid-dependent NS	2
Steroid-resistant NS	21

Serum albumin level, platelet count, and platelet aggregation values are shown in **Table 2**. Data on serum albumin level and platelet aggregation by way of ADP concentrations of 5  $\mu$ M, 2.5 $\mu$ M, and 1  $\mu$ M were not normally distributed (P<0.05), while data on platelet count and platelet aggregation value of 10  $\mu$ M ADP concentration were normally distributed. A typical linear pattern of platelet aggregation and ADP concentration were observed in this study.

**Table 3** shows the correlation between variables

studied. We noted that age and albumin had a significant positive correlation ( $r=0.462$ ;  $P=0.008$ ). However, age and platelet aggregation value showed a significant negative correlation ( $r=-0.353$ ;  $P=0.048$ ) with ADP concentration of  $1\ \mu\text{M}$ . Serum albumin and platelet count had a significant negative association ( $r=-0.617$ ;  $P<0.001$ ), while albumin and platelet aggregation value with  $1\ \mu\text{M}$  ADP concentration had a significant negative association ( $r=-0.658$ ;  $P<0.001$ ), suggesting that lower serum albumin level was associated with increasing platelet count and platelet aggregation. Partial correlation analysis between serum albumin level and platelet count with age as confounding factor is shown in **Table 4**.

Serum albumin had negative correlations with platelet count and platelet aggregation values assessed with ADP concentration of  $1\ \mu\text{M}$ . According to Guilford correlation criteria, the correlation between serum albumin level and platelet aggregation value

was moderate ( $r>0.6$ ).

**Figure 1** shows the negative correlation between serum albumin and platelet count. There was a negative central trend with R Sq linear of 0.392. Hence, lower serum albumin was correlated with higher platelet count. **Figure 2** shows the negative correlation between serum albumin and platelet aggregation. There was a negative central trend with R Sq linear of 0.357. Hence, lower serum albumin was correlated with higher platelet aggregation.

## Discussion

The majority of study subjects were male (18 boys; 56%); the male: female ratio was 1.3:1. A previous study also noted that NS was more common in males compared to females.<sup>12</sup> The mean age of subjects' was 109 months (9 years and 1 month old), which was

**Table 2.** Serum albumin level, platelet count, and platelet aggregation in pediatric NS patients

Variables	Mean (SD)	Median (range)	P value
Albumin serum, g/dL	2.06 (1.23)	2.20 (0.4-4.10)	0.015*
Platelet count, /mm <sup>3</sup>	453,062.5 (187,443.90)	410,000 (160,000-1,022,000)	0.079
Platelet aggregation assessed by ADP agonist, %			
10 $\mu\text{M}$	86.8 (8.63)	87.55 (68-99.70)	0.080
5 $\mu\text{M}$	82.4 (15.33)	85.80 (33.70-101.30)	0.002*
2.5 $\mu\text{M}$	66.6 (24.90)	70.65 (14.60-100.70)	0.002*
1 $\mu\text{M}$	34.95(31.69)	19.95 (0.70-99.60)	<0.001*

Note: \* Shapiro-Wilk test

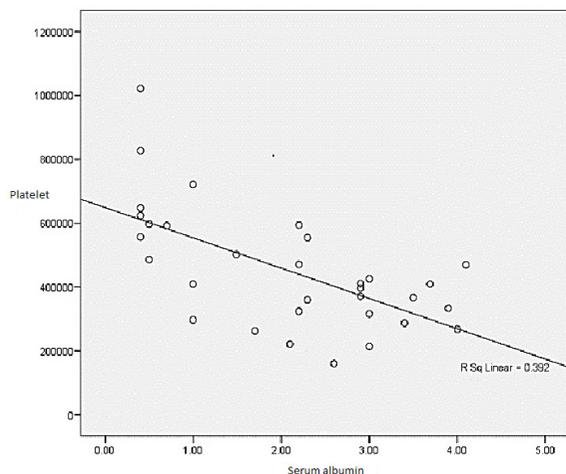
**Table 3.** Analysis of possible correlations between study variables

Variables	Correlation coefficient ( $r_s$ )	P value
Age and albumin	0.462	0.008
Age and platelet count	-0.253	0.163
Age and platelet aggregation		
10 $\mu\text{M}$	0.156	0.395
5 $\mu\text{M}$	0.087	0.636
2.5 $\mu\text{M}$	-0.023	0.899
1 $\mu\text{M}$	-0.353	0.048
Albumin and platelet count	-0.617	<0.001
Albumin and platelet aggregation assessed by ADP agonist		
10 $\mu\text{M}$	-0.139	0.448
5 $\mu\text{M}$	-0.196	0.283
2.5 $\mu\text{M}$	-0.281	0.120
1 $\mu\text{M}$	-0.658	<0.001

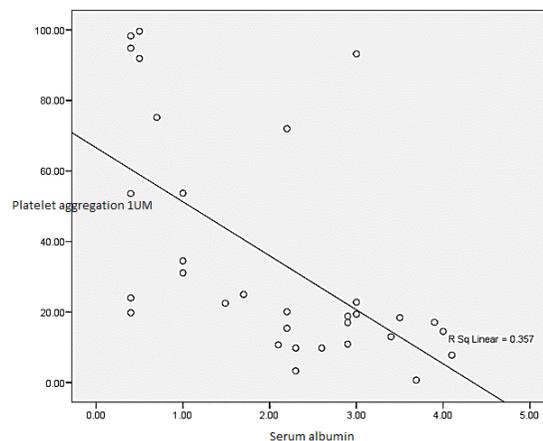
Note:  $r_s$  = Rank Spearman correlation coefficient

**Table 4.** Partial correlation analyses between serum albumin level and platelet count as well as platelet aggregation, with age as a confounding variable

Correlation	Correlation coefficient	P value
Albumin and platelet count	-0.641	<0.001
Albumin and platelet aggregation assessed by ADP agonist concentration of $1\ \mu\text{M}$	-0.634	<0.001



**Figure 1.** Correlation between serum albumin level and platelet count in pediatric NS patients



**Figure 2.** Correlation between serum albumin level and platelet aggregation value, assessed using ADP concentration of 1  $\mu$ M in pediatric NS patients

consistent with the theory that NS is more commonly found among school children and teenagers. Most of our subjects were diagnosed with steroid-resistant NS, which suggests that Dr. Hasan Sadikin General Hospital status is a top referral hospital.

Decreased serum albumin level is one of laboratory criteria needed to establish a diagnosis of nephrotic syndrome according the *International Study of Kidney Disease in Children* (ISKDC), with cutoff point of 2.5 g/dL.<sup>13</sup> All subjects had at least +2 proteinuria on three urinalyses, based on urine dipstick measurement. Those with marked hypoalbuminemia were noted.

Several previous studies found the high platelet count among NS patients with high platelet count.<sup>14-17</sup> Our subjects had a high mean platelet count of 453,062.50 (SD 187,443.90)/mm<sup>3</sup>, ranging between 160,000–1,022,000/mm<sup>3</sup>, while 25% of patients had normal platelet count. An Indian study stated that thrombocytosis was always happened among NS patients, with mean (SD) platelet level of 307,000 (49,099.5)/mm<sup>3</sup>.<sup>7</sup> Mitall *et al.* found that platelet counts in steroid-sensitive NS and focal segmental glomerulosclerosis (FSGS) NS patients were higher than the control. Similarly, our subjects' mean platelet count was markedly high.

All subjects had received steroid medication, with a range of 1 day to 9 years, none of whom reported remission. Platelet count during remission and after cessation of therapy remained high,

but returned to the normal range after long-term remission.<sup>16</sup> The mechanism of elevated platelet count in NS patients is unclear, but several hypotheses suggest links to hypoalbuminemia, hypovolemia, and hypercholesterolemia.<sup>18</sup>

We noted hyperaggregation of platelet in our subjects. An Indian study in 2013 showed increased serum levels of collagen, ristocetin, arachidonic acid, and platelet aggregation rate as assessed by 5 and 2.5  $\mu$ M ADP concentration tests in 29 pediatric NS patients.<sup>7</sup> Patients with NS experience arachidonic acid (AA) release, which metabolizes into thromboxan A<sub>2</sub>, in turn inducing hyperaggregation of platelets. This rising aggregation has been connected to hypoalbuminemia, since albumin may inhibit platelet aggregation induced by arachidonic acid and its conversion.

Statistical analysis revealed that serum albumin level correlated in a linear manner with platelet count (correlation coefficient 0.641;  $P < 0.001$ ). Furthermore, statistical analysis of serum albumin level and platelet aggregation value with ADP concentration of 1  $\mu$ M revealed a moderate coefficient ( $r = -0.634$ ;  $P < 0.001$ ). Hence, serum albumin had negative correlations with those two variables. A previous retrospective study showed that NS patients with proteinuria and hypoalbuminemia had 8 times higher risk of a venous thromboembolic event.<sup>19</sup> Another study on NS patients also reported that serum albumin level of 3-3.99 g/dL would increase the thromboembolic event

risk by 1.5 times, while serum albumin level of 2.5-2.99 g/dL would increase the thromboembolic event risk by 2.2 times, and serum albumin level below 2.5 g/dL would increase the risk by 2.79 times.<sup>11</sup>

This study had several limitations. The sample size was considered small for assessing correlations between these variables. We also did not analyze other factors that might have contributed to thromboembolic events, such as anticoagulation factors, fibrinolytic factors, factors V, VII, VIII, and X, von Willebrand factor, and total serum protein level. Further studies should be conducted with a larger sample size and include an evaluation of a practical diagnostic value of albumin for thromboembolic events.

In conclusion, serum albumin level negatively correlates with platelet count and platelet aggregation among pediatric NS patients.

## Conflict of Interest

Authors stated that there was no competing interest in this study.

## Acknowledgements

The authors received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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