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Original Article

Thrombospondin-1 and blood pressure in 7 to 8-year-old children born low birth weight and small for gestational age

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Abstract

Background Thrombospondin-1 (TSP-1) is associated with endothelial damage, glomerular impairment, and hypertension. Low birth weight (LBW) and small for gestational age (SGA) children have higher risk of morbidity and mortality.

Objective To assess for a possible association between TSP-1 level and blood pressure in children who were born low birth weight and small for gestational age.

Methods We conducted a cross-sectional study from March to May 2015. Inclusion criteria were children who were born LBW and SGA in 2007-2008 at Prof. Dr. R. D. Kandou General Hospital, resided in Manado, North Sulawesi, had complete medical records, and whose parents consented to their participation. Exclusion criteria were children who were in puberty, obese, had renal disease, taking medications that affect blood pressure, or who were admitted to the hospital in the 2 weeks prior to enrollment. Data were analyzed using regression and simple correlation tests to assess for associations between TSP-1 and birth weight, as well as TSP-1 and blood pressure.

Results Subjects' mean TSP-1 level was 257.95 ng/dL. There was a strong negative correlation between TSP-1 and birth weight ($r=-0.784$; $P<0.0001$). In addition, there were strong positive correlations between TSP-1 level and systolic blood pressure ($r=0.718$; $P<0.0001$) as well as TSP-1 and diastolic blood pressure ($r=0.670$; $P<0.0001$).

Conclusion Higher TSP-1 is associated with higher systolic and diastolic blood pressure in 7 to 8-year-old children who were LBW and SGA at birth. Also, TSP-1 and birth weight have a strong negative correlation. **[Paediatr Indones. 2017;57:269-73; doi: http://dx.doi.org/10.14238/pi57.5.2017.269-73]**.

Keywords: *thrombospondin-1; blood pressure; low*

There has recently been an increasing interest in the influence of intrauterine life on the pathogenesis of chronic diseases. Infants who are born SGA have an increased risk of developing hypertension and cardiovascular diseases during adult life. Such conditions include dyslipidemia, glucose intolerance, hyperinsulinemia, and insulin resistance. *The Barker Hypothesis* postulated that these diseases are pre-programmed, due to an inadequate supply of nutrients during fetal development.[1-3] A history of SGA is a predictive factor of high blood pressure in early adulthood.[4-6] There have been many studies on the relationship between SGA and blood pressure, including other factors such as renal volume and function, plasma homocysteine, uric acid, the ACE gene, and P-selectin.[7] Building on the hypothesis, it is increasingly being recognized that an anti-angiogenic state is implicated in the

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pathophysiology of SGA pregnancies. The TSP-1, an inhibitor of angiogenesis, is a cellular matrix protein that was identified during cell injury. This TSP-1 is present in vascular circulation and binds to cellular receptors and other proteins in the vascular structure.[7]

Few studies have been done to determine a direct relationship between TSP-1 level, birth weight, and blood pressure in animals, especially in children born LBW and SGA.[8,9] Therefore, the objective of this study was to assess for possible associations between TSP-1 level and birth weight, as well as TSP-1 and blood pressure in 7 to 8-year-old children who were born LBW and SGA.

Methods

We conducted a cross-sectional study from March to May 2015 in Manado. The study was approved by the Ethics Committee of Sam Ratulangi University Medical School.

Subjects were healthy children who were born LBW and SGA seven to eight years prior to the study (2007-2008) in Prof. Dr. R.D. Kandou Hospital, had complete medical records, resided in Manado, North Sulawesi, and whose parents consented to participation. We excluded children who were in puberty, obese, had renal disease, taking medications that affect blood pressure, or who were admitted to the hospital in the two weeks prior to enrollment. Subjects underwent physical examination as well as anthropometric and blood pressure measurements. Body weight was measured using a platform beam balance scale. Blood pressure was measured in a quiet room in the subject's house, with the subject in a resting position , using a standard sphygmomanometer with an appropriately-sized cuff. Subjects underwent three readings, taken in five-minute intervals. The three readings were averaged to obtain a mean value.

The onset of the first Korotkoff phase was used to determine systolic blood pressure, and the onset of the fifth Korotkoff phase was used to determine diastolic blood pressure. Blood specimens were drawn with aseptic technique. Thrombospondin-1 levels were measured and analyzed using *Human TSP-1 by Quantikinine R&D Systems*.

The correlation between TSP-1 level and blood pressure was analyzed using regression and simple correlation tests. The minimum required sample size was calculated to attain 90% power, and P values < 0.05 were considered to indicate statistical significance. Statistical analysis was performed using *SPSS for Windows version 22.0*.

Results

During the study period, 128 LBW and SGA children were identified. However, 30 children's families could not be reached by phone, 26 had moved away, and 10 parents refused to participate. Hence, the eligible study population was 62 children, but 17 of these were excluded due to lack of cooperation (2), illness (5), and obesity (10). Therefore, 45 subjects were recruited into the study.

Table 1 shows the subjects' baseline characteristics. Subjects' mean birth weight are 2,000 was 1,980g and mean TSP-1 level was 257.95 ng/dL.

Table 1. Baseline characteristics of subjects

Characteristics	N=45
Gender, n(%)	
Male	18 (40)
Female	27 (60)
Age, n(%)	
7 years	29 (64.4)
8 years	16 (35.6)
Mean birth weight (SD), g	1,980 (148.24)
Mean TSP-1 (SD), ng/dL	257.95 (67.13)
Mean systolic BP (SD), mmHg	102.89 (9.91)
Mean diastolic BP (SD), mmHg	68.56 (8.02)

Figures 1, 2, and **3** show the relationships between TSP-1 levels and birth weight, as well as TSP-1 and systolic and diastolic blood pressures, respectively. There was a strong negative correlation between TSP-1 level and birth weight ($r=-0.784$; $P<0.0001$), a strong correlation between TSP-1 level and systolic blood pressure ($r=0.718$; $P<0.0001$), and a strong correlation between TSP-1 level and diastolic blood pressure ($r=0.670$; $P<0.0001$) in children with a history of LBW and SGA.

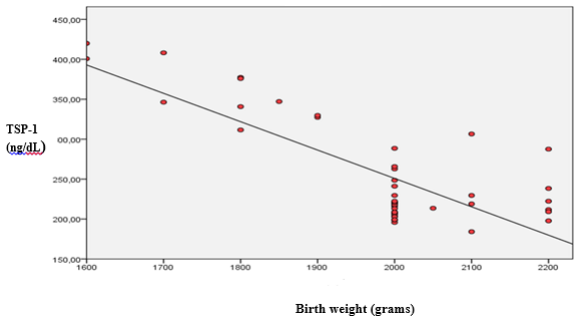

Figure 1. Thrombospondin-1 level (ng/dL) and birth weight (grams)

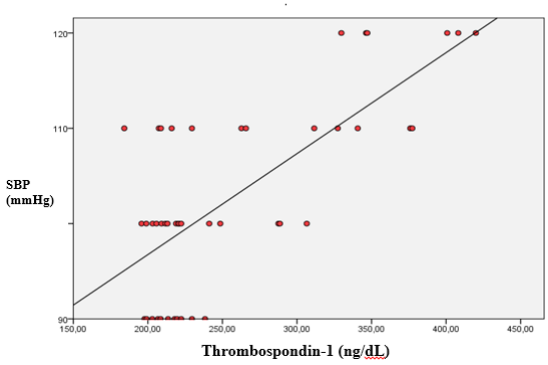

Figure 2. Thrombospondin-1 level and systolic blood pressure

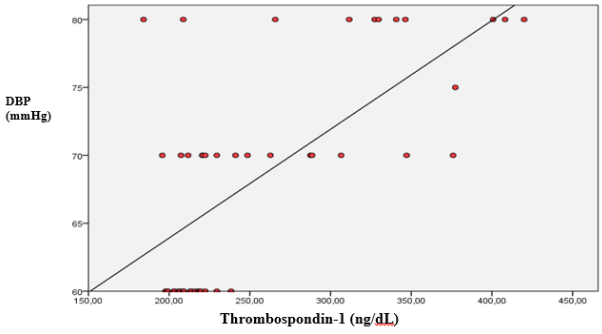

Figure 3. Thrombospondin-1 level and diastolic blood pressure

Discussion

To the best of our knowledge, this is the first study to investigate TSP-1 levels and blood pressure in children with a history of LBW and SGA. A previous study in an animal model determined that blood pressure increased due to several factors, including arterial tone, and increased TSP-1 expression. Our findings suggest that the higher TSP-1 levels correlate to higher systolic and diastolic blood pressures.

Strong positive correlations between TSP-1 level and systolic blood pressure ($r=0.718$; $P<0.0001$) and diastolic blood pressure ($r=0.670$; $P<0.0001$) were statistically significant. Our findings are consistent with those of Bauer *et al*.[8] They measured blood pressure with a transducer implanted in the tails of mice and found a positive correlation between TSP-1 level and blood pressure. Circulating TSP-1 inhibits endothelial nitric oxide synthase (eNOS) activation in endothelial cells. TSP-1-null mouse endothelial cells have inherently greater eNOS activity. Thus, TSP-1 limits production of the diffusible vasodilator nitric oxide (NO). Addition of intravenous TSP-1 to TSP-1-null mice stimulated vasoconstriction that can increase blood pressure. Higher TSP-1 circulating in blood correlated with increased blood pressure.[8]

Another study reported that in a rat model, TSP-1 level could increase diastolic blood pressure and mean arterial pressure (MAP). The integrity of the capillary wall was influenced by the level of TSP-1 circulating in blood. This result demonstrated for the first time that a matricellular protein acutely regulated blood pressure and cardiovascular responses to stress. Therefore, TSP-1 might act either as a direct vasoconstrictor or as an inhibitor of an endogenous vasodilator such as NO.[9]

Circulating TSP-1 is related to the risk of renal disease, especially hypertension in later life, along with other possible mediators that may disturb biomarker pathways (related to oxidative stress, endothelial dysfunction, angiogenesis, capillary rarefaction, and transforming growth factor β1). The TSP-1 enhances fibrosis and renal damage by its interaction with the biomarkers mentioned above . This TSP-1 is expressed in glomerulopathies and is considered an early marker of inflammation and fibrosis. Increased TSP-1 can inhibit NO, that in turn causes vasoconstriction, significantly increased glomerulosclerosis, glomerular matrix accumulation, podocyte injury, renal infiltration with inflammatory cells, and altered renal function parameters.[10-15]

Nevertheless, a comparison between human and animal models of TSP-1 levels and blood pressure should be viewed cautiously since TSP-1 levels and blood pressure in animal models may differ from human models.

We also found that TSP-1 levels in children with history of LBW and SGA were high, as well as

a negative correlation between TSP-1 level and birth weight (r=-0.784; P<0.0001). Similarly, previous publications showed that birth weight was a risk factor for increased TSP-1 levels.[16,17]

Isenberg *et al*. reported that the expression of TSP-1 in wild type mice born SGA and aged between 14 to 18 months demonstrated greater degrees of ischemia in the glomeruli and tubulointerstitium of the kidney and formed atherosclerosis in the vessel walls. TSP-1 level was related to birth weight. Also, TSP-1 increased in response to cellular injury. TSP-1 binding to CD47 receptors inhibits NO signaling by preventing cGMP synthesis and activation of its target cGMP-dependent protein kinase. The CD47 is a ubiquitously-expressed transmembrane protein that is present on all cardiovascular cell types and platelets. This potent antagonism of NO signaling allows TSP-1 to acutely constrict blood vessels, accelerate platelet aggregation, and if sustained, inhibit angiogenic responses.[16]

Andraweera et al. found that increased TSP-1 levels in SGA infants increase the risk of cardiovascular and renal disease in later life. They noted TSP-1 to be a prothrombotic and anti-angiogenic glycoprotein expressed in blood vessels. A single nucleotide polymorphism in the TSP-1 gene (TSP-1 2210A/G) was reported to be a significant risk factor for familial premature myocardial infarction, hypertension, and renal disease, which was associated with SGA, suggesting that TSP-1 polymorphism may be associated with the risk of vascular disorders across the course of life.[17]

In a rat study, TSP-1 expression was more prominent, especially in peritubular interstitial space. The percentage of glomeruli with positive intraglomerular TSP-1 staining was 19.3 (SD4.5) %; (P<0.0001). Tubulointerstitial TSP-1 expression in the cortex was 8.8 (SD 4.2) tubules/mm^2. TSP-1 has been shown to both inhibit endothelial cell proliferation and accelerate endothelial cell death (apoptosis). In addition, Kang et al. found that age-related TSP-1 expression was increased in both glomeruli and interstitium in rat kidneys. The increased TSP-1 expression strongly correlated with the degree of glomerulosclerosis, tubulointerstitial fibrosis, and glomerular hypertension.[18]

Accumulations of TSP-1 in vascular tissue may increase the blood pressure at a younger age, especially in children with a history of LBW and SGA, as related to intrauterine retardation. Even from the time of conception, TSP-1 may affect inhibition of vascular vasodilation and apoptosis of glomerular membrane.[6,7,9,15]

A limitation of this study was that the blood pressure measurements were performed on only one occasion. A 24-hour ambulatory measurement of blood pressure would be more accurate. Another limitation was that TSP-1 was measured only once, hence, further study is needed to monitor the TSP-1 levels in subjects.

In conclusion, 7 to 8-year-old children who were low birth weight and small for gestational age at birth have negatively correlated birth weight and TSP-1 level. Also, higher TSP-1 level is associated with higher blood pressure.

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Conflict of Interest

None declared.

References

1. Goldenberg RL, Culhane JF. Low birth weight in the United States. Am J Clin Nutr. 2007;85:584-90.
2. Villar J, Belizan JM. The relative contribution of prematurity and fetal growth retardation to low birth weight in developing and developed societies. Am J Obstet Gynecol. 1982;143:793-8.
3. Wardlaw T, Blanc A, Zupan J, Ahman E. Low birthweight: country, regional and global estimates. Geneva: WHO Publications; 2004. p.5-6.
4. Gunardi H. Pemantauan bayi prematur. In: Trihono PP, Purnamawati S, Sjarif DR, Hegar B, Gunardi H, Oswari H, editors. Hot topics in pediatrics II. Naskah lengkap Pendidikan Kedokteran Berkelanjutan Ilmu Kesehatan Anak XLV; 2002 Feb 18-19; Jakarta: Balai Penerbit FKUI; 2002. p. 17-27.
5. Hartanto S, Mustadjab I. Profil bayi berat lahir rendah di ruang perinatology RSUP Manado. Batam: Proceedings from

the PIT IKA 11-IDAI; 2004 Jul 12-14; 2004. p. 42.
6. Barker DJ. The developmental origins of adult disease. J Am Coll Nutr. 2004;23:588-95.
7. Isenberg JS, Wink DA, Roberts DD. Thrombospondin-1 antagonizes nitric oxide-stimulated vascular smooth muscle cell responses. Cardiovasc Res. 2006;71;785-93.
8. Bauer EM, Qin Y, Miller T, Bandle R, Csanyi G, Pagano PJ, *et al.* Thrombospondin-1 supports blood pressure by limiting eNOS activation and endothelial-dependent vasorelaxation. Cardiovasc Res. 2010;88:471-81.
9. Isenberg JS, Qin Y, Maxhimer JB, Sipes J, Despres D, Schnermann J, *et al*. Thrombospondin-1 and CD47 regulate blood pressure and cardiac responses to vasoactive stress. Matrix Biol. 2009;28:110-19.
10. Lopez-Dee Z, Pidcock K, Gutierrez LS. Thrombospondin 1: multiple paths to inflammation. Mediators Inflamm. 2011:1-10.
11. Vanhoutte D, Heyman S. Thrombospondin 1: A protective "matric-cellular" signal in the stressed heart. J Hypertens. 2011;58:770-1.
12. Murphy-Ullrich JE, Sage EH. Revisting the matricellular concept. Matrix Biol. 2014;37:1-14.
13. Soto-Pantoja DR, Shih HB, Maxhimer JB, Cook KL, Ghosh A, Isenberg JS, *et al.* Thrombospondin-1 and CD47 signaling regulate healing of thermal injury in mice. Matrix Biol. 2014;37:25-34.
14. Duquette M, Nadler M, Okuhara D, Thompson J, Shuttleworth, Lawler J. Members of the thrombospondin gene family bind stromal interaction molecule 1 and regulate calcium channel activity. Matrix Biol. 2014;37:15-24.
15. Rogers NM, Sharifi-Sanjani M, Csanyi G, Pagano PJ, Isenberg J. Thrombospondin-1 dan CD47 regulation of cardiac, pulmonary and vascular responses in health and disease. Matrix Biol. 2014;37:92-101.
16. Isenberg JS, Frazier WA, Roberts DD. Thrombospondin-1 is a central regulator of nitric oxide signaling in vascular physiology. Cell Mol Life Sci. 2008;65:728-42.
17. Andraweera PH, Dekker A, Thompson SD, North RA, McCowan ME, Roberts CT. A functional variant in the thrombospondin 1 gene and the risk of small for gestational age infants. J Thromb Haemost. 2011;9:2221-8.
18. Kang DH, Anderson S, Kim YG, Mazzalli M, Suga S, Jefferson A, *et al*. Impaired angiogenesis in the aging kidney: vascular endothelial growth factor and thrombospondin-1 in renal disease. Am J Kidney Dis. 2001;37:601-11.