

Endothelial Dysfunction in the Young Adult: a Retrospective Cohort Study on the Effect of Low Birth Weight

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ABSTRAK

Tujuan: untuk mengetahui efek bayi berat lahir rendah (BBLR) terhadap fungsi endotel dan menentukan peran adiponektin plasma pada disfungsi endotel melalui pemeriksaan flow mediated brachial artery (FMBA) atau respon vasodilatasi (VR) dan mengukur kadar asymmetrical dimethylarginine (ADMA) plasma subjek dewasa muda dengan riwayat BBLR. **Metode:** pada penelitian kohort retrospektif, subjek diambil secara acak dari suatu growth study cohort Kecamatan Tanjungsari Kabupaten Sumedang Jawa Barat. Mereka terdiri atas 67 subjek dengan riwayat BBLR dan 67 subjek dengan riwayat bayi berat lahir normal (BBLN). Variabel terikat adalah kadar adiponektin plasma, ADMA plasma dan nilai VR. Uji yang digunakan untuk mengetahui korelasi antara adiponektin plasma dan ADMA adalah korelasi Pearson. **Hasil:** kelompok BBLR secara bermakna mempunyai VR yang lebih rendah, RR=2,94 dengan 95% CI=1,91-4,53; dan kadar adiponektin plasma rendah RR =1,53 dengan 95% CI =1,07-2,18. Seluruh variabel (plasma adiponektin, plasma ADMA dan nilai VR) antara kelompok BBLR dan BBLN menunjukkan perbedaan bermakna pada 2 variabel yaitu nilai VR ($p < 0,001$, 95% CI= -4,409-(-2,114) dan kadar adiponektin plasma yang lebih rendah ($p = 0,015$, 95% CI= -1,083-(-0,082) pada kelompok BBLR dibandingkan kelompok BBLN. Tidak ada korelasi antara kadar adiponektin plasma dan kadar ADMA plasma, maupun antara kadar adiponektin plasma dan nilai VR. **Kesimpulan:** BBLR berefek pada fungsi endotel. BBLR dibandingkan dengan BBLN memiliki nilai VR dan adiponektin plasma yang lebih rendah. Adiponektin plasma berperan kecil pada disfungsi endotel subjek dewasa muda dengan riwayat BBLR.

Kata kunci: BBLR, adiponektin, ADMA, pemeriksaan FMBA.

ABSTRACT

Aim: to investigate the effect of low birth weight (LBW) on endothelial function, and to determine the role of plasma adiponectin in endothelial dysfunction by conducting flow mediated brachial artery (FMBA) test or vasodilation response (VR) and by measuring plasma asymmetrical dimethylarginine (ADMA) of young adults born with LBW. **Methods:** in a retrospective cohort study, subjects were randomly selected from the growth study cohort of Tanjungsari Sumedang district West Java. They consisted of 67 LBW and 67 NBW (Normal Birth Weight) young adults. Dependent variables were plasma adiponectin, plasma ADMA, and VR. The correlation between plasma adiponectin and ADMA level was examined using Pearson's correlation. **Results:** the relative risk for LBW to have low brachialis artery vasodilation response was 2.94, (95% CI: 1.91-4.53), and to have low of plasma adiponectin concentration 1.53, (95% CI: 1.07-2.18). There was a statistically

significant difference for all variables studied (FMBA, plasma ADMA, and plasma Adiponectin concentrations), while simultaneous confidence interval measurements indicated that the value of FMBA and the concentration of plasma adiponectin were significantly lower, respectively $p < 0.001$, 95% CI: -4.409-(-2.114), and $p = 0.015$, 95% CI: -1.083-(-0.082) in LBW compared to NBW subjects. The correlation between plasma adiponectin concentration and plasma ADMA concentration in LBW subjects was not significant. **Conclusion:** there is an effect of LBW on endothelial function. LBW compared to NBW subjects have lower VR and plasma adiponectin concentration. There may be a small role of plasma adiponectin in endothelial dysfunction of young adults with LBW.

Key words: low birth weight, adiponectin, ADMA, FMBA test.

INTRODUCTION

Low birth weight (LBW) may increase the risk of cardiovascular diseases during adulthood. Intrauterine growth restriction can lead to alteration of organ, function, and metabolism and also to persistent endocrine disorders.¹ Adiponectin is a protein that is synthesized by fat cells, and play an important role in fetal growth.^{2,3} In LBW, the low adiponectin concentration persists through the childhood and adulthood.^{4,5} Apart from insulin sensitizer effect, adiponectin has also antithrombotic and antisclerotic properties, that directly influence the endothelial function.⁶

Endothelial dysfunction is a foundation for atherosclerosis. One of the endothelial functions which will first be disturbed is vasodilation and vasoconstriction balance. Nitric oxide (NO) is one of vasodilators produced by the endothelial cells, which has a very short half-time of 3-5 seconds, and can be clinically detected as a vasodilatation response (VR) on the flow mediated brachial artery (FMBA) test⁷⁻⁹ Asymmetric dimethylarginine (ADMA) is a laboratory marker of endothelial dysfunction that acts as an endogen inhibitor of endothel nitric oxide synthase (eNOS) an enzyme that competes with arginine, which is a NO precursor.¹⁰

LBW incidence in Indonesia is still high of about 11.5%.¹¹ Therefore, the aim of this study is to define the effect of LBW on endothelial function represented by VR value and plasma ADMA level; and to determine the role of adiponectin on endothelial dysfunction represented by ADMA level.

METHODS

This was a clinical epidemiological study conducted in a retrospective fashion, from November 2009 until January 2010. Subjects were obtained from the growth study cohort of Tanjungsari, Sumedang District, West Java¹² who fulfilled the inclusion criteria (**Figure 1**).

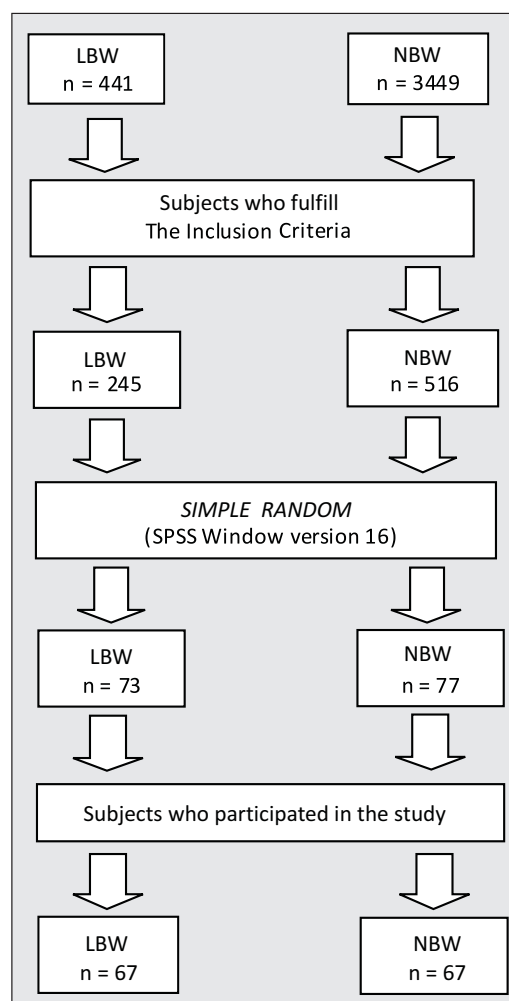


Figure 1. Subjects obtained from the growth study cohort of Tanjungsari, Sumedang District, West Java

LBW as a risk compared with control group born with NBW, which had been identified from 19 to 21 years ago. The outcome were plasma adiponectin, plasma ADMA, and brachial artery VR.

Inclusion criteria included the following: males or females, ranging in age from 19 until 21 years old, had birth weight and length data, had taken part in previous risk factor study, and had been previously randomized. Patients were excluded if one of the following occurred: i) chronic renal failure, ii) liver disease, iii) heart failure, iv) antidiabetic drug, nitrate drug, steroid drug and multivitamin consumption.

In this study, chronic renal failure was defined as chronic kidney disease stages 3,4 and 5 with glomerular filtration rate (GFR) <59, <29 and <15 mL/minute per 1.73 m².¹³ Liver disease was defined as liver cell injury with the elevation of alanine aminotransferase (ALT) >1000 U/L, or at least 300 U/L.¹⁴ Epidemiology criteria of the Framingham Study was used to consider heart failure which met two majors or one major criteria plus two minors criteria.¹⁵

Ultrasonography General Electric logic was used to obtain the diameter of brachial artery. Diameter of brachial artery was measured before and within the first minute after occlusion. VR value (in %) is the differences of diameter of brachial artery after and before occlusion.^{9,16}

Human adiponectin operates based on the underlying principle of a sandwich enzyme-linked immunosorbent assay (ELISA) that uses two kinds of anti-human adiponectin monoclonal antibodies (MoAbs). The specimens are pretreated, total adiponectin and individual multimers of adiponectin are determined selectively, directly or indirectly. Reference value: male 2.54-6.06 µg/mL, female 3.58-9.66 µg/mL. Determine the absorbance of each well at a wavelength of 492 nm with a plate reader. Reference value: male 2.54-6.06 µg/mL, female 3.58-9.66 µg/mL.

The competitive ADMA-ELISA uses the microtiter plate format. ADMA is bound to the solid phase of the microtiter plate. ADMA in the samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit

anti-ADMA antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase ADMA is detected by anti-rabbit/peroxidase. The substrate/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase ADMA is inversely proportional to the ADMA concentration of the sample (expected values: 0.4-0.75 µmol/L).

This study approved by The Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital-Faculty of Medicine Universitas Padjadjaran, Bandung.

Statistical Analysis

The cut-off points were low plasma adiponectin concentration, high plasma ADMA concentration, and low brachialis artery VR value which were determined by diagnostic testing using the receiver operating characteristics (ROC) curves.

Normality testing of data distribution was conducted before completing the statistical analysis using the Lilliefors significance correction. The correlation between plasma adiponectin and ADMA level was examined using Pearson's correlation if the data had normal distribution. The subject characteristic data were stated in mean value and standard deviation (SD). All statistical analysis were conducted using the SPSS window software version 13.0. The values of p<0.05 were considered statistically significant.

RESULTS

There were 67 LBW subjects and 67 NBW subjects enrolled in this study. None of the patients in each group received anti diabetic, antihypertensive, nitrate, vasoactive drugs and/or multivitamin. The general characteristics of two groups were not significantly different (**Table 1**).

The Association between LBW and Concentrations of VR, Plasma Adiponectin, and Plasma ADMA

Cut-off point for low plasma adiponectin concentration from the receiving operating characteristic (ROC) curve is ≤4.13 µg/ml

Table 1. General characteristics of young adults born with LBW and NBW

| Parameters | LBW (n=67) | NBW (n=67) |
|---|--------------|--------------|
| Age, years old | 20.3 (0.5) | 20.2 (0.58) |
| Male, % | 52.2 | 56.7 |
| SBP, mmHg | 117 (10) | 119 (10) |
| DBP, mmHg | 76 (6) | 78 (7) |
| Hemoglobin, gr% | 15.0 (1.6) | 15.3(1.3) |
| Fasting blood glucose, mg/dL | 86.0 (6.7) | 84.8 (8.7) |
| Total cholesterol, mg/dL | 158.5 (26.7) | 161.3 (30.1) |
| Triglycerid, mg/dL | 84.4 (40.4) | 95.10(51.4) |
| HDL-C, mg/dL | 44.4 (8.8) | 43.5 (8.5) |
| LDL-C, mg/dL | 100.1 (23.6) | 101.8(24.6) |
| SGOT, unit/litre | 21.6 (6.5) | 21.5 (7.0) |
| SGPT, unit/litre | 21.5 (13.6) | 20.4 (10.9) |
| Blood urea, mg/dL | 19.1 (5.4) | 18.3 (4.5) |
| Creatinine, mg/dL | 0.68 (0.14) | 0.71 (0.16) |
| Smoker: male, % | 88.6 | 89.5 |
| BMI, kg/M ² | 20.1 (3.3) | 20.7 (3.2) |
| Diabetes Mellitus | None | None |
| Hypertension (BP \geq 140/90 mmHg), n | 1 | 3 |

BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure

and low VR value is \leq 9.5%, while for high plasma ADMA concentration is \geq 0.89 mMol/L. Significantly, the LBW groups are prone to have low VR and low plasma adiponectin than NBW group (**Table 2**).

The Mean Plasma Adiponectin Concentration, Plasma ADMA Concentration and VR Differences in Young Adults Born with LBW and NBW

All three variables showed very significant differences according to the statistical testing using t-test for two independent samples. The next statistical testing using the simultaneous confidence interval found significantly different mean variables of plasma adiponectin concentration and VR value between LBW and NBW. The baseline distribution of Brachialis artery diameter in both groups was not significantly different, but VR value showed significant difference between both groups. The mean plasma ADMA concentration of LBW was not significantly different ($p=0.669$) compared with the NBW group. (**Table 3**)

Table 2. Relative risk for LBW to have low VR, low plasma adiponectin concentration, and high plasma ADMA concentration (n=67)

| Variables | Adiponectin (μ g/ml) | | p | RR (95%CI) | VR (%) | | p | RR (95%CI) | ADMA (μ Mol/L) | | p | RR (95%CI) |
|-----------|---------------------------|------------|---|------------|------------|-----------|---|------------|---------------------|------------|---|------------|
| | \leq 4.13 | >4.13 | | | \leq 9.5 | >9.5 | | | \geq 0.89 | <0.89 | | |
| | LBW | 41 (60.3%) | | | 26 (39.4%) | 0.016 | | | 1.53 (1.07-2.18) | 50 (74.6) | | |
| NBW | 27 (39.7%) | 40 (60.6%) | | | 17 (25.4) | 50 (74.6) | | | 7 (36.8%) | 60 (52.2%) | | |

Note: Chi square test, VR = vasodilation response, RR = relative risk for LBW

Table 3. The mean plasma adiponectin concentration, plasma ADMA concentration, and brachialis artery VR differences between young adults born with LBW and NBW

| Variables | LBW (n=67) | NBW (n=67) | p | 95% CI for difference |
|--|-------------|--------------|---------|-----------------------|
| Plasma Adiponectin, μ g/ml | 4.07 (1.29) | 4.64 (1.61) | 0.015* | -1.083-(-0.082)* |
| Brachialis Artery VR, % | 8.40 (3.06) | 11.66 (3.63) | <0.001* | -4.409-(-2.114)* |
| Baseline diameter of Brachialis Artery, Cm | 3.35 (0.47) | 3.33 (0.42) | 0.758** | |
| Plasma ADMA, μ Mol/L | 0.71 (0.15) | 0.69 (0.15) | 0.669* | -0.041-(-0.064)* |

* Simultaneous confidence interval, ** Independent t-test

Correlation Between Plasma Adiponectin, and Plasma ADMA, and Brachialis Artery VR in Young Adult Subject Born with LBW

There were no correlation between plasma adiponectin and plasma ADMA ($R = -0.16$, $p = 0.176$), and between plasma adiponectin and VR ($R = 0.13$, $p = 0.281$) in LBW. Significant correlation was found between baseline diameter of Brachial artery and VR ($R = 0.27$, $p = 0.028$), with smaller Brachial artery diameter associated with bigger VR.

DISCUSSION

Main finding of this study was that VR significantly differ between LBW and NBW groups, although the baseline diameter of Brachialis artery in both groups were similar.

The endothelial dysfunction was clinically found more in the young adult group born with LBW compared with NBW that appeared as low VR (8.4% vs 11.6%) on FMBA test. Leeson et al found that low VR in LBW was a result of vascular function alteration relating to in-utero growth disturbance.¹⁶

Poor nutrition during fetal growth will result in the alteration of the organ structure and function, also in the metabolism disturbance that appear as LBW with low plasma adiponectin concentration. Adiponectin raises the NO production through eNOS enzyme stimulation. Plasma adiponectin concentration in this study was significantly lower in LBW compared with NBW group. This is similar to the result of study by Kamoda et al (2001)⁴ and Cianfarani et al (2004).⁵ Unlike our study, they compared the Small for Gestational Age group (birth weight = 2.1 ± 0.4 kg) and Appropriate for Gestational Age group (birth weight = 3.0 ± 0.8 kg). In this study, both groups (LBW and NBW) had same BMI (about 20 kg/M²). We could not eliminate the low plasma adiponectin concentration in LBW as the result of fatty tissue malfunction according to Barker & Hales' study¹ and as a result of genetic factor like single nucleotid polymorphisms 276 on Adiponectin gene,⁶ therefore further studies are needed.

In this study, no significant correlation between plasma adiponectin and VR, as well as plasma adiponectin and plasma ADMA were

found. Adiponectin might have some effect on endothelial dysfunction of young adults with LBW.

Other factors might have influenced the final results which precludes us from drawing definite conclusions, before thorough evaluation.

There are a variety of vascular or endothelial functions, however vasodilation and vasoconstriction balance is affected earliest.¹⁷ In addition to the NO vasodilator synthesis disturbance, the potential role of excessive vasoconstrictors in LBW subjects such as endothelin-1, angiotensin II, thromboxan A₂, and prostagladine H₂ deserve further investigation.

The mean plasma ADMA concentration of LBW was not significantly different compared with the NBW. Only few studies have examined the protein arginine N-methyltransferase (PRMT) activity regulation. The study by Osanai et al (2003)¹⁸ found that ADMA concentrations were influenced by shear stress. In physiologic state, shear stress increases the PRMT-1 expression and ADMA synthesis through NF- κ B path activation. However, in the presence of shear stress, the dimethylarginine dimethylamin hydrolase-II (DDAH-II) activity would also increase resulting in ADMA degradation. In this study, the blood samples for ADMA examination were obtained before conducting of FMBA test, thus preventing any differences between the two groups. Oxidative stress that results in ADMA accumulation¹⁹ could be eliminated if only the general characteristics of both groups were similar eg. smoking, systolic and diastolic blood pressure, blood glucose concentration, and blood fat concentration between LBW and NBW groups.

One factor that could influence plasma Adiponectin and ADMA is TNF α . Tumor necrosis factor α inhibits the Adiponectin production,²⁰⁻²² but induces the ADMA accumulation.²³ The possibility of fatty tissue function disturbance¹, especially in TNF α synthesis which is strictly related to adiponectin production and ADMA accumulation, cannot be ruled out.

The low plasma adiponectin concentration and endothelial dysfunction condition persist through the childhood and into adulthood periods. A nonsignificant correlation between

plasma adiponectin and either plasma ADMA concentration, and VR revealed no potential role of adiponectin in endothelial dysfunction in LBW. There might be other factors in cellular level that influence the endothelial function.

Finally, beyond small sample size, other potential limitation are the involvement of intrauterine growth retardation (IUGR), and preterm subjects in LBW group. The findings of this report therefore need further confirmation in future studies with adequately powered.

CONCLUSION

Low birth weight modulates endothelial function. LBW compared to NBW subjects have lower VR and plasma adiponectin concentration. There may be a small role of plasma adiponectin in endothelial dysfunction of young adults with LBW; however other variables might have important role and further studies are needed before definite conclusions regarding plasma adiponectin could be drawn.

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